VOLUME 32 OCTOBER 1954 NUMBER 5

Canadian Journal of Zoology

Editor: E. HORNE CRAIGIE

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF ZOOLOGY

(Formerly Section D, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues annually THE CANADIAN JOURNAL OF ZOOLOGY and six other journals devoted to the publication of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Zoology, National Research Council, Ottawa 2, Canada.
(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover))

Proof, correspondence concerning proof, and *orders for reprints* should be sent to the Manager, Editorial Office (Research Journals), Division of Administration, National Research Council, Ottawa 2, Canada.

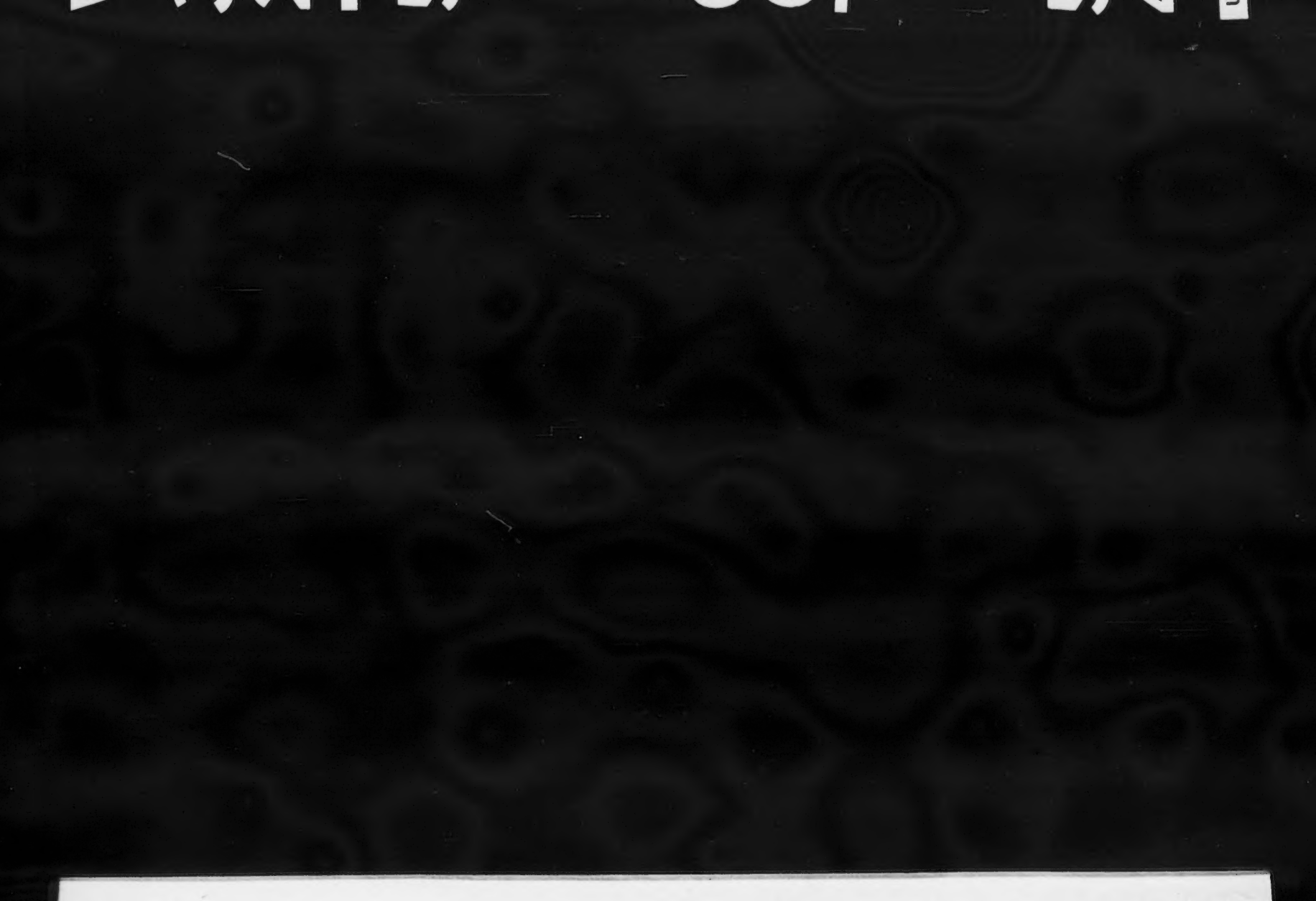
Subscriptions, renewals, and orders for single or back numbers should be sent to Division of Administration, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Bimonthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00
Canadian Journal of Chemistry	Monthly	$5.00
Canadian Journal of Microbiology*	Bimonthly	$3.00
Canadian Journal of Physics	Monthly	$4.00
Canadian Journal of Technology	Bimonthly	$3.00
Canadian Journal of Zoology	Bimonthly	$3.00

The price of single numbers of all journals is 75 cents.

** Volume 1 will combine three numbers published in 1954 with six published in 1955 and will be available at the regular annual subscription rate of $3.00.*



Canadian Journal of Zoology

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 32 OCTOBER 1954 NUMBER 5

NUTRITIONAL STUDIES WITH *PSEUDOSARCOPHAGA AFFINIS* (FALL.), A DIPTEROUS PARASITE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

I. A CHEMICALLY DEFINED MEDIUM AND ASEPTIC-CULTURE TECHNIQUE[1]

By H. L. HOUSE[2]

Abstract

A chemically defined medium consisting mainly of amino acids, dextrose, salts, and vitamins, and an aseptic technique are described for nutritional studies with larvae of *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). In feeding tests with 542 larvae, microorganisms contaminated only 3.9% of the initial number. Within an assay period of 20 days, 83.9% of the aseptic larvae reared on the medium reached the third instar. After removal from the rearing medium, 59.9% of the aseptic larvae pupated and a number of adults emerged. The time required for 50% of the aseptic larvae to develop to the third instar was 9.2 days. This is the first medium composed of chemically pure substances, with the exception of agar, to be successfully used for rearing a parasitic, entomophagous insect. Since the intervention of microorganisms can be avoided, a basis is provided for further nutritional studies with *P. affinis*.

Introduction

The variability and complexity of natural foods have made it extremely difficult to use them for determining the ingredients that are utilized by an animal. The technique of feeding animals purified diets has simplified the problem and is finding wide application. Frequently with insects it is feasible and economical to develop and use diets that are essentially chemically defined. A diet composed of exact quantities of chemically pure substances furnishes a medium that can be altered in composition to satisfy the requisites of most nutritional studies. By the use of such diets the fundamental principles of insect nutrition are being clarified. Variations in the food requirements and in the anatomy and physiology of the various species may modify the application of these natural laws, but they do not invalidate the principles themselves.

Many aspects of insect nutrition have received attention. These aspects have been reviewed by several authors (Uvarov (23); Wigglesworth (26); Craig and Hoskins (4); Trager (20); Brues (3); and Trager (21)). Noteworthy progress has been made in elucidating the chemical nature of factors

[1] *Manuscript received May 19, 1954.*
Contribution No. 3205, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Canada.
[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

[The August number of this Journal (Can. J. Zoology, 32 : 283-330. 1954) was issued July 30, 1954.]

necessary for growth and development. It is recognized that insects require proteins, carbohydrates, sterols, salts, and vitamins of the water-soluble *B* group, nutrients that do not differ significantly from those utilized by the higher vertebrates. Many studies dealing with insect nutrition have been carried on in the presence of microorganisms. As the food may be altered by the activities of these organisms, it is desirable to eliminate them from the experimental animals and their food whenever possible.

Several workers have used aseptic-culture techniques with insects. In 1911, Delcourt and Guyénot (5), using *Drosophila* sp., were apparently the first to rear an insect free from microorganisms; and later other workers succeeded, using other species. Usually the eggs of the insect are treated with a disinfectant and the larvae emerging from them are reared on sterile food. In this way the blow fly *Calliphora vomitoria* (L.) (Wollman (29)), the flesh fly *Phaenicia sericata* (Mg.) (Hobson (9)), the mosquito *Aedes aegypti* (L.) (Trager (19)), and the cockroach *Blattella germanica* (L.) (Wollman (30)) have been reared aseptically. The technique of culturing maggots aseptically for surgical use was widely used and many methods of propagation have been described (e.g., Robinson (17); White (25)).

The most significant recent achievement in the study of insect nutrition has been the development of chemically defined diets capable of supporting the growth of insects in the absence of microorganisms. Schultz, St. Lawrence, and Newmeyer (18) were the first to report on the complete development of an insect, *Drosophila melanogaster* Mg., under aseptic conditions on a diet of known composition. Villee and Bissell (24) studied the effects of substituting nucleotides and purine or pyrimidine bases for ribonucleic acid on the growth of *D. melanogaster* and used a chemical diet similar to that of Schultz, St. Lawrence, and Newmeyer (18). Golberg and De Meillon (7) used a food medium that was almost chemically defined to study the amino acid requirements of *A. aegypti*. Trager (22) used a diet of defined composition to study the activity of biotin and fat-soluble materials in the nutrition of mosquito larvae. In 1949, House (10) reported a diet that was chemically defined and suitable for nutritional studies with *B. germanica*. Begg and Robertson (1) described attempts to develop a chemically defined medium that would support normal growth of *D. melanogaster*. Recently, Kadner and La Fleur (14) used a medium of known vitamin content to determine the vitamin requirements of *P. sericata*.

Synthetic diets composed of exact quantities of known chemical substances promise to provide a basis for fundamental nutritional studies with insect parasites. Preliminary studies were undertaken at the Belleville laboratory with *Pseudosarcophaga affinis* (Fall.). This species is a parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.), and of other insects in Canada (Wilkes (27); Wilkes, Coppel, and Mathers (28); and McGugan (16)). In 1948, House and Traer (13) reported an artificial food consisting of liver and fish for rearing *P. affinis* and described its use in the laboratory rearing of this species.

This paper is a report on a chemically defined diet and a technique for rearing an entomophagous insect, *P. affinis*, aseptically, and on growth and development of the insect under sterile rearing conditions.

Chemically Defined Diet

After repeated trials, a medium was formulated from exact quantities of substances of known composition (Table I). A mixture of amino acids (Table II) comparable in number and relative quantities to those present in casein is substituted for protein. The composition of the amino acids was based on data compiled by Block and Bolling (2) and on an insect diet devised by House (10). Where the racemic form of several amino acids was used it seemed advisable to double the quantity, thereby supplying the *l* form in greater concentration. To avoid a possible deficiency, the quantities of glycine and of cystine were increased. Cystine was used in preliminary work, but cysteine was substituted later, with no apparent ill effect.

The preparation of the medium presents no difficulties, although several techniques are used to prepare the different components. To eliminate microorganisms during preparation, the medium is prepared in two parts, a different technique being used for each. One part contains all the ingredients except the vitamins and dextrose; the other, a solution of vitamins and dextrose.

To prepare the first part, the required quantities of amino acids and salt mixture (e.g., No. 2, U.S.P. XII) are carefully weighed and transferred to a 250-ml. flask containing 30 ml. of distilled water. The mixture is heated gently to dissolve the salts and amino acids.

The other ingredients of part one are prepared separately and are added in turn. A suspension of cholesterol is prepared by mixing 5 gm. of cholesterol in a minimal quantity of 95% ethyl alcohol, and then adding the suspension to 0.25 gm. of polyoxyethylene sorbitan monooleate (Tween 80, Atlas Powder Co. Ltd. of Canada, Brantford, Ontario) in sufficient distilled water to make 100 ml. The result is a milky white suspension of finely dispersed cholesterol, which is added to the previous ingredients by means of a pipette at 0.1 gm. of cholesterol per 2 ml. of Tween solution.

Ribonucleic acid is dissolved in sodium hydroxide before it is added to the other components. A solution of 2 ml. of 2 *N* sodium hydroxide and 7 ml. of distilled water is prepared. This is poured over 0.1 gm. of ribonucleic acid and shaken until the nucleic acid is dissolved. The final solution is then added to the other ingredients. Sodium hydroxide is included to dissolve the nucleic acid and serves also to adjust the pH of the medium to a final value of approximately 5.8. It is important to adjust the pH at this stage, before the agar solution is added, to preserve the gelling properties of the agar.

A solution of agar is prepared at a greater concentration than is required in the complete medium. This solution is made by dissolving 0.75 gm. of agar in 39 ml. of distilled water, resulting in an approximately 2% solution of agar, which is diluted by the other liquid fractions to a final concentration of 0.75%.

For part two, an aqueous solution of vitamins and dextrose is prepared and sterilized. The required quantity of each entity is weighed and dissolved in 20 ml. of distilled water. A fresh solution is prepared immediately before use and, after measures have been taken to eliminate microorganisms, it is added to the other sterilized components.

TABLE I

COMPOSITION OF A CHEMICALLY DEFINED MEDIUM FOR REARING THE DIPTEROUS PARASITE *P. affinis*

Constituent	Concentration, mgm. per ml. of water	Constituent	Concentration, μgm. per ml. of water
Amino acid mixture (Table II)	20.0	Thiamine	5.0
Ribonucleic acid	1.0	Riboflavin	15.0
Cholesterol	1.0	Pyridoxine	15.0
Dextrose	20.0	Inositol	100.0
Salt mixture No. 2 (U.S.P. XII)	2.0	Nicotinic acid	15.0
Agar	7.5	Choline chloride	100.0
		Calcium pantothenate	15.0
		Para-aminobenzoic acid	15.0
		Folic acid	5.0
		Biotin	0.025

TABLE II

COMPOSITION OF THE MIXTURE OF AMINO ACIDS

Amino acid	Concentration, mgm. per ml. of water
dl-Alanine	1.29
l-Arginine*	0.53
dl-Aspartic acid	1.53
l-Cysteine†	0.18
l-Glutamic acid	2.74
Glycine	0.35
l-Histidine*	0.35
l-Hydroxyproline	0.24
dl-Isoleucine	1.53
l-Leucine	1.40
l-Lysine*	0.88
dl-Methionine	0.94
dl-Phenylalanine	1.40
l-Proline	1.00
dl-Serine	1.76
dl-Threonine	0.94
dl-Tryptophane	0.47
l-Tyrosine	0.82
dl-Valine	1.65
Total	20.00

* *Monohydrochloride.*
† *Or cystine.*

Aseptic Technique

Each part of the medium is sterilized separately by means of recognized bacteriological techniques. The first part, containing the more stable ingredients, is autoclaved for 20 min. at 15 lb. pressure. All instruments, apparatus, and glassware used in the aseptic technique are sterilized similarly in the autoclave, or in the dry-air oven for two hours at 180° C., and precautions are taken to prevent recontamination. The second part of the medium is filtered through a Seitz filter to remove microorganisms. Both parts of the medium are placed in a special chamber (House and Patton (12)) where they can be united in an environment relatively free from dust and other sources of contamination. When the autoclaved part of the medium has cooled somewhat, but before the agar gels, 20 ml. of the filtered solution of vitamins and dextrose are added with a pipette and the flask is shaken gently to mix both fractions. This step completes the preparation and, if proper precautions have been taken, results in 100 ml. of sterile medium.

The medium is dispensed while warm into small test tubes, which serve as simple cages for larval rearing. A number of test tubes, about $\frac{3}{8}$ in. in diameter and 3 in. in length, previously plugged with nonabsorbent cotton and sterilized, are placed in the aseptic chamber. Each test tube is unplugged to receive 1 ml. of medium dispensed by means of an automatic pipette. The test tubes with their stoppers replaced are laid in a slanting position until the agar gels. These small test tubes, containing a sloping surface of sterile medium, provide conditions in which each larva can be reared aseptically and in which they may be readily observed throughout an assay.

Large stock cultures of *P. affinis* reared on an artificial diet of liver and fish (House and Traer (13); House (11)) and propagated through several generations in the Belleville laboratory provided gravid females from which larvae were obtained. Precautions, however, were taken to obtain bacteriologically sterile larvae for each assay of the medium. Since *P. affinis* is viviparous, it is necessary to dissect the larvae from gravid females submerged in 2.5% formalin. The larvae obtained in this manner are assisted in escaping from their chorions and are allowed to remain in the formalin for about 5 min. The dish containing the larvae is placed in the special chamber, where the formalin is removed by means of a small pipette. Each larva is transferred on the tip of a sterile brush to a small test tube of sterilized medium, by suitable bacteriological technique to maintain asepsis.

Rearing of *P. affinis*

Usually about 55 test tubes of medium, each containing one larva, were set up for each assay. Tests of the medium were replicated 10 times. All larvae in each experiment were reared in a room with a constant temperature of 23° C. and a relative humidity of approximately 60%. The culture tubes were placed in trays in an almost horizontal position and kept in the dark except during the short periods when they were removed for examination. This practice reduces the tendency of the larvae to move out of their food.

In the transparent medium the larvae were observed closely with a microscope without disturbing them. Any dead larvae found within the first 24 hr. were discarded because death within that period is attributed to causes other than nutritional deficiencies. After the first 24 hr., all deaths were recorded and considered to be a significant response of the larvae to their diet. Each larva was examined daily and its growth was determined by changes in the morphological structure of its mouth parts. Records were maintained for 20 days of the stage of development of each larva, the number of deaths, and the first indication of contamination.

Rearing tubes in which microorganisms were detected are not considered in the final appraisal of the assay. In the earlier assays all the cultures were tested by means of recognized bacteriological media, including proteose tryptone agar, nutritive caseinate agar, potato dextrose agar, and egg–meat medium. Repeated tests with the food medium or macerated larvae for inoculum showed that the food medium itself revealed the presence of microorganisms as readily as the special bacteriological media, and use of the latter was then discontinued except in doubtful cases.

The data were evaluated by a statistical technique devised by Litchfield (15). Only the rate of growth of the larvae was used as a criterion for determining the effects of the medium. At the end of each experiment, however, all the mature larvae were put into moist moss or sawdust, where many of them formed pupae. After a time a few adults emerged, but metamorphosis of the majority was interrupted by diapause. The pupae were collected and stored at 1° C. until diapause was broken. They were then incubated again at room temperature (23° C.) and more adults were usually obtained.

Results

The development of larvae of *P. affinis* reared aseptically on a chemically defined medium is summarized in Table III.

TABLE III

NUMBERS OF LARVAE OF *P. affinis* REARED ASEPTICALLY DURING 20 DAYS ON A CHEMICALLY DEFINED MEDIUM; 10 REPLICATES OF APPROXIMATELY 55 EACH

	Initial number of larvae	Infected	Larvae obtained aseptically						Pupae from aseptic larvae
			Total number	Dead	Survivors failing to attain 3rd instar	Number of larvae reared (exclusive of dead)			
						1st instar	2nd instar	3rd instar	
Total	542	21	521	55	29	486	465	437	312
Percentage of total (542)		3.9	96.1	10.1	5.4	89.7	85.8	80.6	57.6
Percentage of aseptic larvae (521)			—	10.6	5.6	93.3	89.3	83.9	59.9

Table III shows that a successful diet has been developed for studies with this parasite. Of the 521 aseptic larvae, 83.9% reached the third instar and survived the assay period. Also, 59.9% of the aseptic larvae, or 71.4% of

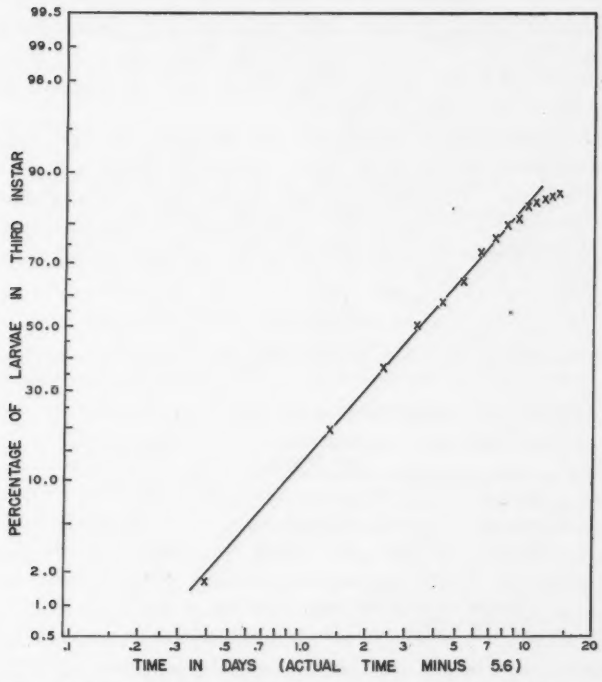


FIG. 1. Cumulative percentages of 521 larvae reared aseptically that attained the third instar, by days.

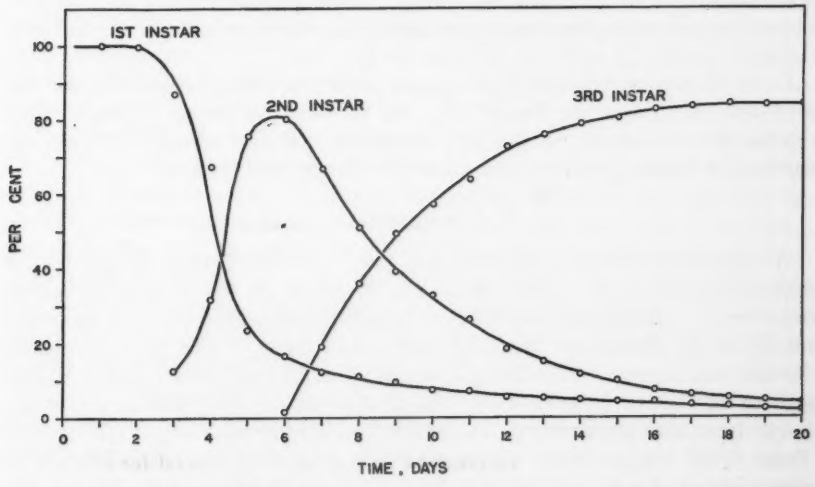


FIG. 2. Rate of development of 521 larvae reared aseptically, shown as the percentages of larvae present by instar by days.

the mature larvae, formed pupae. The pupae were of normal size and shape, 25 averaging 6.6 mm. ± 0.7 mm.* across the long axis and 2.9 ± 0.4 mm. across the short axis in comparison with 6.9 ± 0.4 mm. and 3.0 ± 0.4 mm. respectively for 25 from stock cultures.

The rate of maturation of the larvae is shown in Fig. 1. Trial showed that 5.6 subtracted from each time in days permitted a straight line to be fitted to the plotted data. The median effective time (ET_{50}) of the appearance of mature larvae was 9.2 days, 8.9–9.5 days being the 95% confidence limits.

Table IV gives the average durations of the first and second instars for the aseptic larvae.

TABLE IV

AVERAGE DURATIONS IN DAYS OF FIRST AND SECOND INSTARS OF LARVAE REARED ASEPTICALLY ON A CHEMICALLY DEFINED MEDIUM

Average	First instar (476 larvae)	Second instar (446 larvae)
Mode	5.5	3.5
Median	5.3 (3–19)§	4.8 (1–13)§
Mean	5.5 ± 1.5†	5.3 ± 2.2†

§ *Extreme limits of the range.*
† *Standard error.*

Fig. 2 indicates the changes in the entire population of aseptic larvae throughout the assay period. Four to five days passed before 50% of the larvae reached the second instar, and about nine days before 50% of the original number reached the third instar. The change between the first and second instars took place more abruptly than that between the second and third.

Large flies were obtained from pupae of larvae reared aseptically on the medium. To date, 31.7% of the pupae have produced adults. This represents a relatively small adult emergence, partly because of the poorer survival of males; females outnumbered males by two to one.

Discussion

A chemically defined medium has been developed that is capable of supporting growth of a parasitic insect, *P. affinis*, in the absence of micro-organisms. The medium, consisting of 19 amino acids, 10 B-complex vitamins, nucleic acid, cholesterol, dextrose, salts, and agar, is the first chemically defined diet reported for rearing a parasitic, entomophagous insect. Larval growth on the synthetic medium is somewhat slower than that on either the insect host, *C. fumiferana*, or an artificial food of animal origin (House and Traer (13); House (11)). A reduced rate of growth has been observed in other species of insects on similar chemical diets (Schultz, St. Lawrence, and

* *Standard error.*

Newmeyer (18); Trager (22); House (10)). Nevertheless, the present diet permits the maturation of *P. affinis* and provides a basic medium for continuing research on the nutrition of parasites.

In the present study, the rate of growth of the larvae provides the most reliable criterion for evaluating the efficiency of the medium. Critical appraisal of the results on complete development is not feasible because numerous variable conditions that are difficult to control are introduced by diapause and the subsequent long period of storage in moist sawdust at low temperature. Nevertheless, the formation of both pupae and flies adds significance to the results. Of the aseptic larvae, 59.9% formed pupae. House and Traer (13) reported that of larvae of *P. affinis* reared nonaseptically on the prepupae of the budworm only 37.5% formed pupae; and of larvae reared on liver and salmon, 88.0%.

The adults obtained in the present work were large, well-formed specimens, indicating that the larval medium approximates the requirements for normal development to the adult. More females than males completed development on the medium, 66% females being obtained as compared with approximately 48% from larvae reared on the liver and fish medium. These results indicate that the medium more generally meets the nutritive requirements of the female than those of the male. Working with the cockroach *Blattella germanica*, Hilchey (8) has shown that the amino acid requirements differ for male and female nymphs. To date, matings of male and female flies reared have not resulted in fertilized eggs. The gonads of both sexes, however, showed no apparent abnormality.

The efficiency of the diet for growth and development may possibly be increased by changes in both the quantitative and the qualitative composition of the medium. Many of the ingredients are very likely not included at optimum concentrations. Other experiments showed that a medium containing 5% casein hydrolyzate supported larval growth at a more rapid and uniform rate than the present medium, which contains a mixture of amino acids approximately equivalent to casein, but only at the level of 2% of the diet. The use of racemic mixtures of amino acids could not always be avoided, although Schultz, St. Lawrence, and Newmeyer (18) pointed out that these are toxic to *Drosophila melanogaster*. Since the present study was undertaken, slightly greater survival and more rapid, uniform growth were obtained when other lipids in addition to cholesterol were included in the diet. It has also been found that many of the amino acids are essential for growth. It is likely that a medium with greater amounts of some of these or the other nutrients would beneficially affect larval growth and development.

Growth on the chemically defined medium does not depend entirely on the chemical composition of the diet. Physical properties of the medium contributed toward the readiness with which the larvae consumed the food. The medium was most attractive to the very young larvae when the concentration of agar was low. Young larvae tended to wander from a firmly set agar gel, although older larvae were able to burrow into the gel easily. On the other

hand, a medium containing no agar was not suitable, even when cotton fibers were incorporated into it to support the larvae on the surface. Also, young larvae stayed on the food more readily when it was allowed to slope along the side of the tube to provide a larger surface. Prolonged exposure to light caused the larvae to interrupt their feeding and wander about the inside of the test tube. Growth was more uniform when the physical conditions were satisfactory to the larvae. It appears that the rearing tubes are unsatisfactory environments for pupation and consequently development is delayed. However, the majority of the mature larvae formed pupae within a day or two after being placed in moist moss or sawdust.

Cholesterol frequently caused considerable mortality among the larvae reared on the preliminary media. In the earlier work cholesterol was mixed into the medium by the method of Trager (22). This was moderately successful with media containing casein, but when the medium contained mixtures of amino acids in solution the majority of the larvae died. The larvae had become encrusted with cholesterol, especially around their posterior spiracles. The difficulty was overcome by adding an emulsifying agent, polyoxyethylene sorbitan monooleate (Tween 80), to the medium. This modified the physical properties of the medium so that large particles of cholesterol no longer collected and adhered to the bodies of the larvae. Golberg and De Meillon (6) reported that cholesterol in solution had no beneficial effect on mosquito larvae, but was beneficial when adsorbed on the other components. Work in progress at the Belleville laboratory, however, shows that cholesterol in fine suspension has a beneficial effect on the growth of *P. affinis*.

Contamination occurred in approximately 4% of the culture tubes. When attempts were made to rear the larvae aseptically en masse, the cultures usually became contaminated with bacteria and less frequently with molds. Contamination did not appear to affect the growth of larvae except where putrefaction reduced the medium to a more liquid state or where molds became very dense.

A basic diet having been devised, the effects of dietary constituents on the growth and development of an entomophagous insect can now be studied precisely by omitting or adding nutrients. This work also makes available a constant, definable medium for exact studies on a parasitic species concerning other biological problems in which the results may vary with the nutritional state of the animal. The present medium, or one slightly modified for a specific purpose, would be useful in genetic experiments in view of the evidence that certain chemical compounds control the expression of some genes. Similarly, variation in the expression of metabolic and more precisely physiological activities of an insect often can be attributed to variation in nutrition. In investigations on the biological control of injurious insects, fundamental studies on the physiology of parasites have been largely neglected. Defined chemical diets provide a useful tool for solving many basic problems in research with parasitic species.

References

1. BEGG, M. and ROBERTSON, F. W. The nutritional requirements of *Drosophila melanogaster*. J. Exptl. Biol. 26 : 380-387. 1950.
2. BLOCK, R. J. and BOLLING, D. The amino acid composition of proteins and foods: analytical methods and results. Charles C. Thomas, Publisher, Springfield, Ill. 1945.
3. BRUES, C. T. Insect dietary. Harvard Univ. Press, Cambridge, Mass. 1946.
4. CRAIG, R. and HOSKINS, W. M. Insect biochemistry. Ann. Rev. Biochem. 9 : 617-640. 1940.
5. DELCOURT, A. and GUYÉNOT, E. Génétique et milieu. Nécessité de la determination des conditions. Sa possibilité chez les Drosophiles. Bull. sci. France et Belg. 45 : 249-333. 1911.
6. GOLBERG, L. and DE MEILLON, B. The nutrition of the larvae of *Aedes aegypti* Linnaeus. 3. Lipid requirements. Biochem. J. (London), 43 : 372-379. 1948.
7. GOLBERG, L. and DE MEILLON, B. The nutrition of the larvae of *Aedes aegypti* Linnaeus. 4. Protein and amino acid requirements. Biochem. J. (London), 43 : 379-387. 1948.
8. HILCHEY, J. D. Studies on the qualitative requirements of *Blattella germanica* (L.) for amino acids under aseptic conditions. Contribs. Boyce Thompson Inst. 17 (3) : 203-219. 1953.
9. HOBSON, R. P. Studies on the nutrition of blowfly larvae. IV. The normal role of microorganisms in larval growth. J. Exptl. Biol. 9 : 366-377. 1932.
10. HOUSE, H. L. Nutritional studies with *Blattella germanica* (L.) reared under aseptic conditions. II. A chemically defined diet. Can. Entomologist, 81 : 105-112. 1949.
11. HOUSE, H. L. Notes on the laboratory propagation of *Pseudosarcophaga affinis* (Fall.), a sarcophagid parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). Rept. Quebec Soc. Protection Plants, 31st (1948-49) : 134-137. 1951.
12. HOUSE, H. L. and PATTON, R. L. Nutritional studies with *Blattella germanica* (L.) reared under aseptic conditions. I. Equipment and technique. Can. Entomologist, 81 : 94-100. 1949.
13. HOUSE, H. L. and TRAER, M. G. An artificial food for rearing *Pseudosarcophaga affinis* (Fall.), a parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). Ann. Rept. Entomol. Soc. Ontario, 79th (1948) : 50-53. 1949.
14. KADNER, C. G. and LA FLEUR, F. M. The vitamin requirements of *Phaenicia sericata* (Meigen) larvae (Diptera: Calliphoridae). Wasmann J. Biol. 9 : 129-136. 1951.
15. LITCHFIELD, J. T., JR. A method for rapid graphic solution of time-per cent effect curves. J. Pharmacol. Exptl. Therap. 97 : 399-408. 1949.
16. MCGUGAN, B. M. Insect parasites of the spruce budworm in the Lake Nipigon area of Ontario. Ph.D. Thesis, University of Wisconsin. 1951.
17. ROBINSON, W. The culture of sterile maggots for use in the treatment of osteomyelitis and other suppurative infections. U.S. Dept. Agr. Bur. Entomol. Plant Quarantine, No. E-311. 1933.
18. SCHULTZ, J., ST. LAWRENCE, P., and NEWMEYER, D. A chemically defined medium for the growth of *Drosophila melanogaster*. Anat. Record, 96 : 540. 1946.
19. TRAGER, W. The culture of mosquito larvae free from living microorganisms. Am. J. Hyg. 22 : 18-25. 1935.
20. TRAGER, W. The nutrition of invertebrates. Physiol. Revs. 21 : 1-35. 1941.
21. TRAGER, W. Insect nutrition. Biol. Revs. Cambridge Phil. Soc. 22 : 148-177. 1947.
22. TRAGER, W. Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. J. Biol. Chem. 176 : 1211-1223. 1948.
23. UVAROV, B. P. Insect nutrition and metabolism. A summary of the literature. Trans. Entomol. Soc. London, 76 : 255-343. 1929.
24. VILLEE, C. A. and BISSELL, H. B. Nucleic acids as growth factors in *Drosophila*. J. Biol. Chem. 172 : 59-66. 1948.
25. WHITE, G. F. Rearing maggots for surgical use. *In* Culture methods for invertebrate animals, *Edited by* P. S. Galtsoff *et al.* Comstock Publishing Co., Inc., Ithaca, N.Y. 1937. pp. 418-427.
26. WIGGLESWORTH, V. B. The principles of insect physiology. E. P. Dutton and Co., Inc., New York, N.Y. 1939.
27. WILKES, A. The introduction of insect parasites of the spruce budworm, *Archips fumiferana* Clem. into Eastern Canada. Can. Entomologist, 78 : 82-86. 1946.
28. WILKES, A., COPPEL, H. C., and MATHERS, W. G. Notes on the insect parasites of the spruce budworm, *Choristoneura fumiferana* (Clem.) in British Columbia. Can. Entomologist, 80 : 135-155. 1948.
29. WOLLMAN, E. Sur l'élevage des mouches stériles. Contribution à la connaissance du rôle des microbes dans les voies digestives. Ann. inst. Pasteur, 25 : 79-88. 1911.
30. WOLLMAN, E. Observation sur une ligne aseptique de blattes (*Blattella germanica*) datant de cinq ans. Compt. rend. soc. biol. 95 : 164-165. 1926.

NUTRITIONAL STUDIES WITH *PSEUDOSARCOPHAGA AFFINIS* (FALL.), A DIPTEROUS PARASITE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

II. EFFECTS OF ELEVEN VITAMINS ON GROWTH[1]

By H. L. House[2]

Abstract

When the parasitic dipteron *Pseudosarcophaga affinis* (Fall.) was reared aseptically on chemically defined media; thiamine, riboflavin, calcium pantothenate, nicotinic acid, choline chloride, and biotin were essential for larval growth and development, but not vitamin B_{12}, pyridoxine, folic acid, para-aminobenzoic acid, or inositol. Vitamin B_{12} had a slight beneficial effect on pupation, whereas the omission of para-aminobenzoic acid had a slight stimulatory effect on growth and development. The responses of larvae to deficiencies of different vitamins reached a decisive point at different stages of larval development.

Introduction

Among the first to consider the accessory factors in the nutrition of insects were Guyénot (10), Loeb and Northrop (18), Portier (23), and Wollman (29). Many of the factors have since been identified and are classified today as vitamins. The necessity for specific vitamins in the food of insects and differences in requirements between species have been determined by numerous workers. Trager (27), who has made the most recent comprehensive review of insect nutrition, points out that most of the species investigated require thiamine, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid.

More recent investigations provide additional information. Fraenkel and Blewett (7) reported six species of insects that required folic acid. Leclercq (14, 15) demonstrated that seven vitamins were essential for growth in *Tenebrio molitor* L. A detailed account of the nutritional requirements of this insect is given by Fraenkel, Blewett, and Coles (8). In *Blattella germanica* (L.), the requirement for choline was determined quantitatively by Noland and Baumann (20) and the individual effects of 11 vitamin deficiencies on growth were determined by Noland, Lilly, and Baumann (21).

Much specific knowledge has been obtained from carefully controlled experiments with purified diets. It has been shown that microorganisms, particularly yeasts, can furnish insects with essential food factors that might otherwise be lacking in their diets (Fraenkel and Blewett (4), Blewett and Fraenkel (2), and Pant and Fraenkel (22)). In determining the vitamin requirements of insects there have been a few investigations in which microorganisms were presumably eliminated and the nutrients were chemically

[1] *Manuscript received May 19, 1954.*
Contribution No. 3206, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

defined, at least with reference to vitamins. By such a method, Trager (28) demonstrated the necessity for biotin in the food of *Aedes aegypti* (L.); Kadner and La Fleur (13) determined the vitamin requirements in *Phaenicia sericata* (Mg.); and Hinton, Noyes, and Ellis (11) studied the vitamin requirements of *Drosophila melanogaster* Mg.

The following account deals with the individual effects of 11 vitamins on the growth and development of *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.), reared aseptically throughout its larval stages on chemically defined media.

Material and Methods

A chemically defined medium and an aseptic technique for rearing larvae of *P. affinis*, developed by House (12), provide a means for determining the effects of vitamins on larval growth by altering the composition of the medium by the omission or addition of single vitamins.

The effects of thiamine, riboflavin, pyridoxine, folic acid, para-aminobenzoic acid, pantothenic acid, nicotinic acid, inositol, choline chloride, and biotin on larval growth of *P. affinis* were determined by omitting each vitamin singly from the chemically defined medium (12), the larvae being reared aseptically. The effects of vitamin B_{12} were demonstrated by adding this substance to the basic medium.

Gravid females obtained from stock cultures propagated in the laboratory on liver provided larvae for the present studies. Aseptic larvae were obtained by dissecting them from the females while submerged in 2.5% formalin. After being left in the formalin for about five minutes, the larvae were transferred individually to rearing tubes containing 1 ml. of sterile medium.

The response of the larvae to each vitamin was determined by feeding tests replicated three times, involving a total of over 160 larvae. Larvae obtained from each female were distributed equally between the experimental diet and the control. The larvae were reared individually and were permitted to feed for 20 days in a room with a controlled temperature of 23° C. and a relative humidity of about 60%. Observations were made daily and the stage of development of the larvae was recorded, as well as the numbers of dead larvae and infected cultures. Larvae found in infected media were immediately eliminated from the experiments. Doubtful cases of contamination were tested on regular culture media for detecting microorganisms. The number of larvae attaining the third instar and the time required to reach this stage were the criteria used to compare the effectiveness of the different diets. The data obtained were analyzed by a statistical method for solving time – per cent effect relationships (Litchfield (16), and Litchfield and Wilcoxon (17)).

As an index of larval growth the formula n/t, where n is the percentage of larvae reaching the second or third instar within 20 days and t is the mean time in days required to reach ecdysis, was used whereby the rate of growth may be balanced against the survival of the larvae.

Results

The results obtained in the feeding tests are summarized in Table I.

TABLE I

NUMBERS OF LARVAE OF *P. affinis* REARED ASEPTICALLY DURING 20 DAYS THROUGH VARIOUS INSTARS WHEN VARIOUS VITAMINS WERE REMOVED FROM OR ADDED TO A CHEMICALLY DEFINED MEDIUM; THREE REPLICATES OF APPROXIMATELY 55 EACH

Vitamin*	Total number of larvae												Pupae from aseptic larvae
	First instar				Second instar				Third instar				
	Max.†	Final‡	Dead	Infected	Max.†	Final‡	Dead	Infected	Max.†	Final‡	Dead	Infected	
Thiamine	164	27	17	0	120	84	31	5	0	0	0	0	0
Riboflavin	164	1	21	0	142	93	16	5	28	28	0	0	0
Pyridoxine	164	6	7	1	150	17	3	1	129	121	3	5	115
Folic acid	163	2	4	0	157	17	4	0	136	134	1	1	120
Para-aminobenzoic acid	164	4	4	3	153	6	3	1	143	141	2	0	134
Calcium pantothenate	162	24	104	1	33	15	18	0	0	0	0	0	0
Nicotinic acid	164	86	31	6	41	33	7	1	0	0	0	0	0
Inositol	165	5	5	2	153	9	2	10	132	123	1	8	118
Choline chloride	164	9	20	4	131	102	15	1	13	10	3	0	0
Biotin	161	5	18	4	134	109	4	2	19	19	0	0	0
B_{12}	165	1	17	5	142	20	7	2	113	110	1	2	98

* *B_{12} added; all others removed separately.*

† *Total number of larvae under observation, including those that died or became contaminated, in each instar during the assay.*

‡ *Number of living aseptic larvae present on the final day of the assay.*

Table I shows that on the medium lacking calcium pantothenate none of the larvae developed to the third instar and only a few reached the second. Similar results were obtained on the medium lacking nicotinic acid. With no thiamine in the medium most of the larvae developed to the second instar, although a few reached the third. When riboflavin, choline chloride, or biotin was omitted, growth was slower and only a few larvae reached the third instar.

Table II summarizes the results of a nomographic analysis (16) of the experimental data. The ET_{50} is the median effective time, or the time required for 50% of the larvae to attain the third instar. The slope function (S) is an estimation of individual variation and defines the limits (ET_{50}/S and $ET_{50} \times S$) between which two-thirds of the larvae reached the third instar. Therefore, the greater the value of the slope function the wider is the range of reaction times. Table II shows that there were no significant differences between larvae reared on a control medium and those reared on a medium from which was omitted pyridoxine, folic acid, para-aminobenzoic acid, or inositol. Similarly, there was no significant difference between larvae reared on a medium containing vitamin B_{12} and those on a control medium. With each of these experimental media the median effective time to reach the

third instar was similar. However, when thiamine, riboflavin, calcium pantothenate, nicotinic acid, choline chloride, or biotin was omitted, the median effective time was significantly greater ($P < 0.05$). Tests for parallelism between each experimental medium and a control medium show that the growth curves may be considered parallel within experimental error ($P > 0.05$); hence the larvae on each medium reacted with equal uniformity.

TABLE II

THE PERCENTAGES OF ASEPTIC LARVAE ATTAINING THE THIRD INSTAR AND THE STATISTICAL SIGNIFICANCES OF THEIR RATES OF GROWTH WHEN VARIOUS VITAMINS WERE REMOVED OR ADDED, IN COMPARISON WITH THE CONTROLS

Vitamin	Larvae attaining 3rd instar, %	Slope function* and confidence limits	ET_{50}† and confidence limits, days	Probability P‡	
				Parallelism	Reaction time
Thiamine	0	0	0		
Control	81.4	2.0 (1.8–2.2)	10.8 (10.2–11.4)		< 0.05
Riboflavin	17.6	0	0		
Control	85.4	2.1 (1.9–2.2)	10.2 (9.7–10.8)		< 0.05
Pyridoxine	79.0	2.4 (2.1–2.7)	10.1 (9.5–10.7)		
Control	81.8	2.5 (2.3–2.8)	9.9 (9.3–10.6)	> 0.05	> 0.05
Folic acid	83.3	2.8 (2.5–3.2)	8.9 (8.4– 9.4)		
Control	86.0	2.8 (2.5–3.2)	8.5 (8.1– 8.9)	> 0.05	> 0.05
Para-aminobenzoic acid	89.4	3.0 (2.6–3.4)	8.1 (7.8– 8.5)		
Control	86.0	2.8 (2.5–3.2)	8.5 (8.1– 8.9)	> 0.05	> 0.05
Calcium pantothenate	0	0	0		
Control	85.3	2.4 (2.2–2.7)	9.2 (8.7– 9.8)		< 0.05
Nicotinic acid	0	0	0		
Control	81.8	2.5 (2.3–2.8)	9.9 (9.3–10.6)		< 0.05
Inositol	84.8	2.7 (2.4–3.1)	9.1 (8.6– 9.7)		
Control	81.8	2.5 (2.3–2.8)	9.9 (9.3–10.6)	> 0.05	> 0.05
Choline chloride	8.1	0	0		
Control	86.0	2.8 (2.5–3.2)	8.5 (8.1– 8.9)		< 0.05
Biotin	12.3	0	0		
Control	81.5	2.1 (1.9–2.3)	10.9 (10.3–11.6)		< 0.05
B_{12}§	71.2	2.7 (2.9–3.1)	10.9 (10.1–11.9)		
Control	76.7	2.6 (2.3–2.9)	11.1 (10.4–11.9)	> 0.05	> 0.05

* *Equivalent of the standard deviation.*
† *The time required for 50% of the larvae to attain the third instar.*
‡ *Considered significant when less than 0.05.*
§ *Added; all others removed separately.*

Table III shows the growth index of larvae for both the second and third instars. When growth was most nearly optimum on the chemically defined diet the value was 20 or more for larvae reaching the second instar and 10 or more for those reaching the third.

TABLE III

INDEXES OF GROWTH OF LARVAE DURING 20 DAYS ON ASEPTIC MEDIA WHEN VARIOUS VITAMINS WERE REMOVED OR ADDED

Vitamin	Second instar			Third instar		
	Larvae attaining instar, % n	Average time, t, days	Index n/t	Larvae attaining instar, % n	Average time, t, days	Index n/t
Thiamine	72.3	5.7	12.7	0	0	0
Control	96.3	4.8	20.1	81.4	11.0	7.4
Riboflavin	86.8	5.5	15.8	17.6	16.6	1.1
Control	95.5	4.7	20.3	85.4	10.7	8.0
Pyridoxine	91.1	4.8	19.0	79.0	9.9	8.0
Control	97.8	5.0	19.6	81.8	10.3	7.9
Folic acid	96.3	4.5	21.4	83.3	9.2	9.0
Control	93.9	4.6	20.4	86.0	9.3	9.2
Para-aminobenzoic acid	95.0	4.6	20.6	89.4	8.9	10.0
Control	93.9	4.6	20.4	86.0	9.3	9.2
Calcium pantothenate	20.5	5.7	3.6	0	0	0
Control	95.7	4.7	20.4	85.3	9.6	8.9
Nicotinic acid	25.5	9.3	2.7	0	0	0
Control	97.8	5.0	19.6	81.8	10.3	7.9
Inositol	93.1	5.0	18.6	84.8	9.8	8.6
Control	97.8	5.0	19.6	81.8	10.3	7.9
Choline chloride	84.9	4.8	17.7	8.1	11.5	0.7
Control	93.9	4.6	20.4	86.0	9.3	9.2
Biotin	85.2	6.7	12.7	12.3	17.3	0.7
Control	91.3	4.8	19.0	81.5	10.9	7.5
B_{12}*	88.5	5.3	16.7	71.2	10.9	6.5
Control	93.2	4.9	19.0	76.7	10.7	7.2

* *Added; all others removed separately.*

Discussion

It is concluded that thiamine, calcium pantothenate, nicotinic acid, riboflavin, choline chloride, and biotin are essential dietetic constituents for normal growth and development of larvae of *P. affinis*. Pyridoxine, folic acid, para-aminobenzoic acid, inositol, and vitamin B_{12} are not considered essential since growth was not impeded when they were excluded from the diet.

Though it is generally accepted that insects require thiamine, riboflavin, nicotinic acid, pyridoxine, folic acid, pantothenic acid, and choline, exceptions have been reported. *Oryzaephilus surinamensis* (L.) does not require thiamine or pyridoxine (Fraenkel and Blewett (3, 5)) and neither *Lasioderma serricorne* (F.) nor *Stegobium paniceum* (L.) requires riboflavin, nicotinic acid, or pyridoxine in the presence of microorganisms (Fraenkel and Blewett (4, 5),

Blewett and Fraenkel (2)). Folic acid is not required by *Blattella germanica* and its omission from the diet seems to stimulate growth (Noland, Lilly, and Baumann (21)). Choline appears not to be required in *O. surinamensis* (Fraenkel and Blewett (3, 5)), in *S. paniceum* (Pant and Fraenkel (22)), or in *Tenebrio molitor* (Martin and Hare (19)), although in a more recent study (Fraenkel, Blewett, and Coles (8)) growth of the third insect was somewhat retarded in the absence of choline.

The fact that microorganisms can supply nutrients to insects has often obscured the elucidation of specific dietetic ingredients, particularly vitamins, essential for insects. In larvae of *L. serricorne* and *S. paniceum*, symbiotic yeasts provide sufficient quantities of thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, and folic acid for the larvae to grow normally, or almost so, on synthetic diets entirely lacking in these substances (Fraenkel and Blewett (4), Blewett and Fraenkel (2), and Pant and Fraenkel (22)). Recognition of the role of symbiotic microorganisms in these insects suggests that the nutritional requirements of other species should be reconsidered in order to obtain a more critical determination of vitamin requirements.

In the present work the vitamins required by *P. affinis* closely resemble those necessary for other species of insects reared aseptically. The most striking difference is that neither pyridoxine nor folic acid is required in the diet of *P. affinis*. Pyridoxine is essential for growth or development in defaunated specimens of *L. serricorne* and *S. paniceum* (2, 3, 22) and in aseptically reared specimens of *Drosophila melanogaster* (Tatum (25, 26)), *Aedes aegypti* (Trager (27)), and *P. sericata* (Kadner and La Fleur (13)). Similarly, folic acid is required by *D. melanogaster* (Begg and Robertson (1)), *A. aegypti* (Golberg, De Meillon, and Lavoipierre (9)), and *L. serricorne* and *S. paniceum* (3). Kadner and La Fleur (13) showed, however, that neither folic acid nor biotin is required in the food of *P. sericata*.

Vitamin B_{12} was not among the vitamins required for larval growth by *P. affinis*. Only 71.2% of the aseptic larvae entered the third instar when B_{12} was present in the food, compared to 76.7% on food from which this entity was omitted (Table II). Also, the growth index for larvae (Table III) both at the second and at the third instar was less for the food containing B_{12} than for the control. Vitamin B_{12} may play a minor role in the formation of pupae. In every test a greater percentage of the mature larvae pupated among those that had received B_{12} in their food than among those reared on control diets; for the totals, the percentages were 81.8% and 74.0% respectively. Hinton, Noyes, and Ellis (11) have shown that this vitamin caused a consistent, though small, improvement in larval growth of *D. melanogaster* and an increase in the number of pupae formed. The studies with *D. melanogaster* and with *P. affinis* appear to be the only records of the nutritional importance of B_{12} in insects reared in the absence of microorganisms.

P. affinis larvae do not require inositol or para-aminobenzoic acid for growth. These vitamins have never been recognized as being of great

importance to insects. Fraenkel and Blewett (5) state, however, that inositol and para-aminobenzoic acid appear to have slight nutritional value for *Ptinus tectus* Boield. and (6) that inositol has some beneficial effect on the growth of *Ephestia kühniella* Zell. Growth and development are improved in *D. melanogaster* when para-aminobenzoic acid is omitted from the food (11). Similar results were obtained with *P. affinis*. The omission of para-aminobenzoic acid resulted in greater survival, the greatest number of mature larvae (Table I), and the highest growth index rating (Table III). The rate of growth was not significantly different ($P > 0.05$) from that for the control (Table II).

The omission of riboflavin, choline chloride, or biotin from the food did not prevent a small number of larvae on each of the diets from attaining the third instar. Growth was somewhat erratic on diets lacking these vitamins, particularly riboflavin. The reason for this is not apparent at the present time. Limited biosynthesis may be taking place. Choline requirements possibly could be met, in part, by other dietetic ingredients, such as methionine, particularly with reference to labile methyl groups. Sufficient stores of these vitamins may also be laid down in the egg by the female to permit limited growth in the larvae. Provision of these vitamins by microorganisms can be ruled out in the present work. Hinton, Noyes, and Ellis (11) found that when choline was omitted from the diet of *D. melanogaster* growth proceeded at a normal rate, but no pupation occurred.

The present studies have shown that the reactions of larvae to deficiencies of different vitamins reach a decisive point at different stages of development. The greatest number of larvae died in the first instar or failed to enter the second on media lacking calcium pantothenate or nicotinic acid (Table I). On a medium lacking thiamine many larvae failed to enter the second instar, although most of them did, but it is doubtful whether any of them would have reached the third instar if the assay period had been extended. On the other media that lacked an essential vitamin, the rate of growth was slow, but most of the larvae entered the second instar and, on some diets, a few individuals attained the third instar. This gives a measure of the dependence the larvae have on these vitamins and the stage of development at which the need for each becomes decisive.

A small number of adults were produced from each lot of pupae formed from larvae reared on several of the experimental media. These adults appeared to be normal in size and similar in numbers to those produced on the control medium. Diapause, intervening after the puparium was formed, delayed the emergence of many flies. Much of the mortality is attributed to factors other than nutritional deficiencies. Little significance can be attached, therefore, to differences in the numbers of adults obtained from the various assays.

Stanley (24) has pointed out that the fastest rate of growth may not be the most suitable since it might be accompanied by lessened survival. Therefore, he developed a "relative environmental index" in an attempt to evaluate the rate of development of his insects with their survival. Trager (28) used a

similar method to appraise the growth responses of larvae. Growth indexes in the present work show the relative effectiveness of the diets; hence diets lacking choline chloride or biotin may be considered similar in value for rearing larvae to the third instar, as indicated by growth indexes of 0.7 (Table III). Growth indexes also provide a useful method for comparing the various experimental diets used in the series of nutritional studies in progress at the Belleville laboratory.

References

1. BEGG, M. and ROBERTSON, F. W. The nutritional requirements of *Drosphila melanogaster*. J. Exptl. Biol. 26 : 380-387. 1950.
2. BLEWETT, M. and FRAENKEL, G. Intracellular symbiosis and vitamin requirements of two insects, *Lasioderma serricorne* and *Sitodrepa panicea*. Proc. Roy. Soc. (London), B, 132 : 212-221. 1944.
3. FRAENKEL, G. and BLEWETT, M. Vitamins of the B-group required by insects. Nature, 151 : 703-704. 1943.
4. FRAENKEL, G. and BLEWETT, M. Intracellular symbionts of insects as a source of vitamins. Nature, 152 : 506-507. 1943.
5. FRAENKEL, G. and BLEWETT, M. The vitamin B-complex requirements of several insects. Biochem. J. (London), 37 : 686-692. 1943.
6. FRAENKEL, G. and BLEWETT, M. The dietetics of the caterpillars of three *Ephestia* species, *E. kühniella*, *E. elutella*, and *E. cautella*, and of a closely related species, *Plodia interpunctella*. J. Exptl. Biol. 22 : 162-171. 1946.
7. FRAENKEL, G. and BLEWETT, M. The importance of folic acid and unidentified members of the vitamin B-complex in the nutrition of certain insects. Biochem. J. (London), 41 : 469-475. 1947.
8. FRAENKEL, G., BLEWETT, M., and COLES, M. The nutrition of the mealworm, *Tenebrio molitor* L. (Tenebrionidae: Coleoptera). Physiol. Zoöl. 23 : 92-108. 1950.
9. GOLBERG, L., DE MEILLON, B., and LAVOIPIERRE, M. The nutrition of the larvae of *Aedes aegypti* L. II. Essential water soluble factors from yeast. J. Exptl. Biol. 21 : 90-96. 1945.
10. GUYÉNOT, E. Recherches expérimentales sur la vie aseptique et la développement d'un organisme (*Drosophila ampolophila*) en fonction du milieu. Bull. biol. France et Belg. 51 : 1-330. 1917.
11. HINTON, T., NOYES, D. T., and ELLIS, J. Amino acids and growth factors in a chemically defined medium for *Drosophila*. Physiol. Zoöl. 24 : 335-353. 1951.
12. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). I. A chemically defined medium and aseptic-culture technique. Can. J. Zool. 32 : 331-341. 1954.
13. KADNER, C. G. and LA FLEUR, F. M. The vitamin requirements of *Phaenicia sericata* (Meigen) larvae (Diptera: Calliphoridae). Wasmann J. Biol. 9 : 129-136. 1951.
14. LECLERCQ, J. Sur les besoins nutritifs de la larve de *Tenebrio molitor* L. Biochim. et Biophys. Acta, 2 : 329-332. 1948.
15. LECLERCQ, J. La biotine, facteur de croissance pour *Tenebrio molitor*. Arch. intern. physiol. 57 : 67-70. 1949.
16. LITCHFIELD, J. T., JR. A method for rapid graphic solution to time-per cent effect curves. J. Pharmacol. Exptl. Therap. 97 : 399-408. 1949.
17. LITCHFIELD, J. T., JR. and WILCOXON, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96 : 99-113. 1949.
18. LOEB, J. and NORTHROP, J. H. On the influence of food and temperature upon the duration of life. J. Biol. Chem. 32 : 103-121. 1917.
19. MARTIN, H. E. and HARE, L. The nutritive requirements of *Tenebrio molitor* larvae. Biol. Bull. 83 : 428-437. 1942.
20. NOLAND, J. E. and BAUMANN, C. A. Requirement of the German cockroach for choline and related compounds. Proc. Soc. Exptl. Biol. Med. 70 : 198-201. 1949.
21. NOLAND, J. E., LILLY, J. H., and BAUMANN, C. A. Vitamin requirements of the cockroach *Blatella germanica* (L.) Ann. Entomol. Soc. Amer. 42 : 154-164. 1949.

22. PANT, N. C. and FRAENKEL, G. The function of the symbiotic yeasts of two insect species, *Lasioderma serricorne* F. and *Stegobium* (*Sitodrepa*) *paniceum* L. Science, 112 : 498-500. 1950.

23. PORTIER, P. Développement complet des larves de *Tenebrio molitor*, obtenu au moyen d'une nourriture sterilisée à haute température (130°). Compt. rend. soc. biol. 82 : 59-60. 1919.

24. STANLEY, J. The environmental index, a new parameter as applied to *Tribolium*. Ecology, 27 : 303-314. 1946.

25. TATUM, E. L. Nutritional requirements of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. 25 : 490-497. 1939.

26. TATUM, E. L. Vitamin B requirements of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. 27 : 193-197. 1941.

27. TRAGER, W. Insect nutrition. Biol. Revs. Cambridge Phil. Soc. 22 : 148-177. 1947.

28. TRAGER, W. Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. J. Biol. Chem. 176 : 1211-1223. 1948.

29. WOLLMAN, E. Larves de mouche (*Calliphora vomitoria*) et vitamines. Compt. rend. soc. biol. 82 : 1208-1210. 1919.

NUTRITIONAL STUDIES WITH *PSEUDOSARCOPHAGA AFFINIS* (FALL.), A DIPTEROUS PARASITE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

III. EFFECTS OF NINETEEN AMINO ACIDS ON GROWTH[1]

By H. L. House[2]

Abstract

Feeding tests with the parasite *Pseudosarcophaga affinis* (Fall.) reared aseptically on chemically defined media showed that food lacking *l*-arginine, *l*-histidine, *dl*-isoleucine, *l*-leucine, *l*-lysine, *dl*-methionine, *dl*-phenylalanine, *dl*-threonine, *dl*-tryptophane, or *dl*-valine failed to support larval growth beyond the first instar. Omitting glycine from the diet lowered the rate of growth and permitted only a small number of individuals to develop beyond the first instar. There were statistically significant differences between the rates of growth on the control diets and on diets lacking *dl*-alanine, glycine, *dl*-serine, or *l*-tyrosine. When *dl*-aspartic acid, *l*-cysteine, *l*-glutamic acid, *l*-hydroxyproline, or *l*-proline was omitted from the food, larval growth and development were not affected.

Introduction

Animals depend upon food proteins for adequate supplies of the amino acids that they cannot synthesize. It is not unusual for adults of many species of insects to maintain life for considerable times in the absence of nitrogenous food, but all require proteins or their equivalent amino acids for growth. Insects, like vertebrates, cannot synthesize all the amino acids in sufficient quantities. Some must be supplied from dietetic sources and these are designated the essential amino acids.

Essential amino acids have been determined for a number of insects. Michelbacher, Hoskins, and Herms (14) showed that larvae of *Phaenicia sericata* (Mg.) require cystine. The larvae of *Drosophila melanogaster* Mg. require arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine (Van't Hoog (18), Lafon (9), Hinton, Noyes, and Ellis (5)). The mosquito *Aedes aegypti* (L.) requires a similar number of amino acids for larval growth (Golberg and De Meillon (3)). At least 10 amino acids are essential for growth in the carpet beetle *Attagenus* sp. (Moore (15)), and in *Tribolium confusum* Duv. (Lemonde and Bernard (11)). Cystine is required by larvae of the hide beetle, *Dermestes maculatus* Deg. (Gay (2)). Leclercq (10) has shown that *Tenebrio molitor* L. requires lysine and tryptophane. House (6) has shown that the nymphs of *Blattella germanica* (L.) require valine, tryptophane,

[1] *Manuscript received May 19, 1954.*

Contribution No. 3207, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

histidine, and probably arginine for normal growth when reared aseptically on chemically defined media; and that cystine is probably essential for development. Hilchey (4), using the same techniques, found that alanine, isoleucine, leucine, lysine, proline, and serine are also essential for *B. germanica*, but proline and serine were required only by males. Noland and Baumann (16), using somewhat different techniques, showed that tryptophane and methionine were necessary for rapid growth of *B. germanica*. Recently, Davis (1) found that arginine and tryptophane were essential in *Oryzaephilus surinamensis* (L.); glycine, histidine, isoleucine, methionine, and valine were probably essential; proline, lysine, and alanine were not essential; and cystine probably was not required. There was an interaction between proline and alanine whereby alanine neutralized the retardation of growth caused by proline in the diet. No one appears to have studied the amino acid requirements of an entomophagous insect.

This paper is a report on amino acid requirements for growth and development of *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.).

Materials and Methods

Larvae of *P. affinis* were reared aseptically on chemically defined media as described by House (7). This medium, containing 19 amino acids, served as a control diet in the present work. Effects of the individual amino acids were determined by omitting them singly from the control diet. To maintain a constant quantity of amino acids in all the diets, glutamic acid was added in a weight equal to the constituent withheld. Additional hydroxyproline was used when glutamic acid was omitted.

The techniques used for conducting the assays were similar to those used previously to study the vitamin requirements (House (8)). Triplicate assays were carried out in a room maintained at 23° C. and a relative humidity of 60%. Daily observations were made on each larva for 20 days. Only the rates of growth of the larvae maintained free of microorganisms were considered in the final evaluation of the results.

The data obtained for each deficient diet and its control were compared and the differences between the results were analyzed by means of time – per cent effect relationship (Litchfield and Wilcoxon (13), Litchfield (12)) and a growth index (Trager (17)) obtained from the expression n/t, where n represents the percentage of larvae reaching the second or third instar within 20 days and t is the mean time in days required to reach ecdysis.

Results

A summary of the results obtained from the feeding tests is shown in Table I.

TABLE I

NUMBERS OF LARVAE OF *P. affinis* REARED ASEPTICALLY DURING 20 DAYS THROUGH VARIOUS INSTARS WHEN VARIOUS AMINO ACIDS WERE REMOVED FROM A CHEMICALLY DEFINED MEDIUM; THREE REPLICATES OF APPROXIMATELY 55 EACH

Amino acid	Total number of larvae												Pupae from aseptic larvae
	First instar				Second instar				Third instar				
	Max.*	Final†	Dead	Infected	Max.*	Final†	Dead	Infected	Max.*	Final†	Dead	Infected	
dl-Alanine	166	16	22	5	123	11	2	4	106	106	0	0	77
l-Arginine HCl	165	5	154	6	0	0	0	0	0	0	0	0	0
dl-Aspartic acid	164	4	3	1	156	13	2	1	140	139	1	0	126
l-Cysteine	161	1	6	2	152	5	4	0	143	142	1	0	107
l-Glutamic acid	159	4	5	0	150	13	4	3	130	129	0	1	104
Glycine	161	69	29	9	54	13	4	3	34	33	0	1	13
l-Histidine HCl	162	0	158	4	0	0	0	0	0	0	0	0	0
l-Hydroxyproline	164	1	5	3	155	6	3	8	138	134	0	4	98
dl-Isoleucine	165	3	160	2	0	0	0	0	0	0	0	0	0
l-Leucine	163	10	151	2	0	0	0	0	0	0	0	0	0
l-Lysine HCl	164	3	161	0	0	0	0	0	0	0	0	0	0
dl-Methionine	157	10	146	1	0	0	0	0	0	0	0	0	0
dl-Phenylalanine	163	3	157	3	0	0	0	0	0	0	0	0	0
l-Proline	167	0	12	4	151	9	0	6	136	131	4	1	106
dl-Serine	163	3	29	0	131	16	3	0	112	109	1	2	88
dl-Threonine	161	5	154	2	0	0	0	0	0	0	0	0	0
dl-Tryptophane	162	23	137	2	0	0	0	0	0	0	0	0	0
l-Tyrosine	165	10	29	0	126	12	4	0	110	105	5	0	83
dl-Valine	160	0	160	0	0	0	0	0	0	0	0	0	0

* *Total number of larvae under observation, including those that died or became contaminated, in each instar during the assay.*

† *Number of living aseptic larvae on the final day of the assay.*

Table I clearly shows that larval growth is inhibited when certain amino acids are omitted from the diet. Food lacking *l*-arginine, *l*-histidine, *dl*-isoleucine, *l*-leucine, *l*-lysine, *dl*- methionine, *dl*-phenylalanine, *dl*-threonine, *dl*-tryptophane, or *dl*-valine failed to support larval development beyond the first instar. Omitting glycine lowered the rate of growth and limited development beyond the first instar to only a small number of individuals.

Statistical evaluations of the differences between the results obtained for each deficient diet and its control are shown in Table II. Nomograph analysis showed significant differences in growth ($P < 0.05$) between the results obtained for the control diet and for a diet lacking *dl*-alanine, *l*-arginine, glycine, *l*-histidine, *dl*-isoleucine, *l*-leucine, *l*-lysine, *dl*-methionine, *dl*-phenylalanine, *dl*-serine, *dl*-threonine, *dl*-tryptophane, *l*-tyrosine, or *dl*-valine. Omitting *dl*-alanine, glycine, *dl*-serine, or *l*-tyrosine resulted only in a reduction in the rate of growth, but the remaining 10 of these amino acids were indispensable. When *dl*-aspartic acid, *l*-cysteine, *l*-glutamic acid, *l*-hydroxyproline, or *l*-proline was omitted from the food, larval growth and development were not affected ($P > 0.05$).

TABLE II

THE PERCENTAGES OF ASEPTIC LARVAE ATTAINING THE THIRD INSTAR AND THE STATISTICAL SIGNIFICANCES OF THEIR RATES OF GROWTH WHEN VARIOUS AMINO ACIDS WERE REMOVED, IN COMPARISON WITH THE CONTROLS

Amino acid	Aseptic larvae attaining 3rd instar, %	Slope function* and confidence limits	ET_{50}† and confidence limits, in days	Probability P‡	
				Parallelism	Reaction time
dl-Alanine	67.5	2.7 (2.3–3.1)	13.9 (12.5–15.9)		
Control	85.4	2.6 (2.3–2.9)	10.0 (9.4–10.7)	> 0.05	< 0.05
l-Arginine HCl	0	0	0		
Control	92.8	2.1 (1.9–2.3)	8.3 (8.0– 8.6)		< 0.05
dl-Aspartic acid	86.0	2.3 (2.1–2.5)	8.8 (8.4– 9.2)		
Control	88.0	2.3 (2.1–2.5)	8.8 (8.4– 9.2)	> 0.05	> 0.05
l-Cysteine	89.9	2.9 (2.6–3.3)	8.5 (8.1–9.0)		
Control	91.6	2.9 (2.6–3.3)	8.1 (7.7– 8.6)	> 0.05	> 0.05
l-Glutamic acid	82.2	2.1 (1.9–2.3)	10.0 (9.5–10.5)		
Control	82.4	2.1 (1.9–2.3)	9.7 (9.2–10.2)	> 0.05	> 0.05
Glycine	22.3	0	0		
Control	85.4	2.6 (2.3–2.9)	10.0 (9.4–10.7)		< 0.05
l-Histidine HCl	0	0	0		
Control	92.8	2.0 (1.8–2.2)	8.4 (8.1– 8.7)		< 0.05
l-Hydroxyproline	89.9	2.8 (2.4–3.1)	8.7 (8.2– 9.3)		
Control	92.3	2.8 (2.4–3.1)	8.7 (8.2– 9.3)	> 0.05	> 0.05
dl-Isoleucine	0	0	0		
Control	82.8	3.4 (2.9–3.9)	8.6 (8.1– 9.2)		< 0.05
l-Leucine	0	0	0		
Control	85.4	2.6 (2.3–2.9)	10.0 (9.4–10.7)		< 0.05
l-Lysine HCl	0	0	0		
Control	92.8	2.1 (1.9–2.3)	8.3 (8.0– 8.6)		< 0.05
dl-Methionine	0	0	0		
Control	85.2	2.8 (2.4–3.1)	8.9 (8.4– 9.5)		< 0.05
dl-Phenylalanine	0	0	0		
Control	82.8	3.4 (2.9–3.9)	8.6 (8.1– 9.2)		< 0.05
l-Proline	86.5	2.6 (2.3–2.9)	9.6 (9.2–10.5)		
Control	82.5	2.9 (2.5–3.3)	9.0 (8.5– 9.6)	> 0.05	> 0.05
dl-Serine	68.3	3.1 (2.6–3.6)	12.1 (11.0–13.4)		
Control	88.7	2.4 (2.2–2.7)	8.8 (8.4– 9.3)	< 0.05	< 0.05
dl-Threonine	0	0	0		
Control	90.6	1.8 (1.7–2.0)	8.7 (8.4– 9.1)		< 0.05
dl-Tryptophane	0	0	0		
Control	92.8	2.1 (1.9–2.3)	8.3 (8.0– 8.6)		< 0.05
l-Tyrosine	66.7	2.3 (2.1–2.6)	12.0 (11.2–13.0)		
Control	89.5	2.3 (2.1–2.6)	8.4 (8.1– 8.8)	> 0.05	< 0.05
dl-Valine	0	0	0		
Control	83.6	2.1 (1.9–2.3)	9.6 (9.2–10.1)		< 0.05

* *Equivalent of the standard deviation.*

† *The time required for 50% of the larvae to attain the third instar.*

‡ *Considered significant when less than 0.05.*

The relative performance of each diet in promoting growth is indicated in Table III, by the index values, which decrease as the effectiveness of the diets decrease.

TABLE III

INDEXES OF GROWTH OF LARVAE DURING 20 DAYS ON ASEPTIC MEDIA WHEN VARIOUS AMINO ACIDS WERE REMOVED

Amino acid	Second instar			Third instar		
	Larvae attaining instar, % n	Average time t, days	Index n/t	Larvae attaining instar, % n	Average time t, days	Index n/t
dl-Alanine	75.9	5.6	13.5	67.5	12.6	5.4
Control	89.9	4.6	19.5	85.4	10.4	8.2
l-Arginine HCl	0	0	0	0	0	0
Control	97.4	4.3	22.6	92.8	8.9	10.4
dl-Aspartic acid	93.9	4.4	21.3	86.0	9.4	9.1
Control	98.1	4.6	21.3	88.0	9.3	9.5
l-Cysteine	95.6	4.6	20.8	89.9	9.4	9.6
Control	93.6	4.6	20.3	91.6	9.1	10.1
l-Glutamic acid	93.6	4.9	19.1	82.2	10.4	7.9
Control	94.3	4.6	20.5	82.4	10.1	8.2
Glycine	33.8	7.2	4.7	22.3	15.3	1.5
Control	89.9	4.6	19.5	85.4	10.4	8.2
l-Histidine HCl	0	0	0	0	0	0
Control	97.4	4.3	22.6	92.8	8.9	10.4
l-Hydroxyproline	96.0	5.4	17.8	89.9	9.5	9.5
Control	94.2	5.0	18.8	92.3	9.6	9.6
dl-Isoleucine	0	0	0	0	0	0
Control	91.1	4.6	19.8	82.8	9.2	9.0
l-Leucine	0	0	0	0	0	0
Control	89.9	4.6	19.5	85.4	10.4	8.2
l-Lysine HCl	0	0	0	0	0	0
Control	97.4	4.3	22.6	92.8	8.9	10.4
dl-Methionine	0	0	0	0	0	0
Control	85.4	5.3	16.1	85.2	8.6	10.8
dl-Phenylalanine	0	0	0	0	0	0
Control	91.1	4.6	19.8	82.8	9.2	9.0
l-Proline	91.0	4.9	18.6	86.5	10.4	8.3
Control	91.9	4.7	19.5	82.5	9.4	8.8
dl-Serine	80.1	5.5	14.6	68.3	10.5	6.5
Control	95.6	4.7	20.3	88.7	9.5	9.3
dl-Threonine	0	0	0	0	0	0
Control	96.6	4.7	20.5	90.6	9.2	9.8
dl-Tryptophane	0	0	0	0	0	0
Control	97.4	4.3	22.6	92.8	8.9	10.4
l-Tyrosine	76.4	5.5	13.9	66.7	10.9	6.1
Control	95.6	4.4	21.7	89.5	9.1	9.8
dl-Valine	0	0	0	0	0	0
Control	93.1	4.8	19.4	83.6	9.5	8.8

Discussion

The present work is believed to be the first study of the dietetic requirements for amino acids in parasitic entomophagous insects. Triplicate assays of diets lacking individual amino acids and their respective controls have established that the dietetic requirements of the parasite *P. affinis* for amino acids may be classified into three groups. Absence of any one of the first group, consisting of *l*-arginine, *l*-histidine, *dl*-isoleucine, *l*-leucine, *l*-lysine, *dl*-methionine, *dl*-phenylalanine, *dl*-threonine, *dl*-tryptophane, and *dl*-valine, prevents larval development beyond the first instar. Absence of any one of the second group, consisting of *dl*-alanine, glycine, *dl*-serine, and *l*-tyrosine, reduces the rate of growth and the number of larvae that reach the third instar within 20 days. Absence of any one of the third group, consisting of *dl*-aspartic acid, *l*-cysteine, *l*-glutamic acid, *l*-hydroxyproline, and *l*-proline, does not affect larval growth or development.

The amino acids of the first group are certainly indispensable. Failure of the larvae to grow in the absence of any one of these 10 amino acids indicates that this parasitic species is unable to synthesize the missing amino acid in sufficient quantities.

The requirement of the larvae for the amino acids of the second group, *dl*-alanine, glycine, *dl*-serine, and *l*-tyrosine, is more uncertain. The omission of any one of these from the food results in slower growth and fewer mature larvae. The effect of the omission of glycine is particularly noticeable (Tables I, II, III). Glycine, therefore, may possibly be among the essential amino acids. Generally these four amino acids are dispensable for larval growth of other insects (1, 11, 15). In the diet of *Aedes aegypti*, however, glycine is essential and the absence of tyrosine reduces the rate of growth of the larvae (3). Alanine and serine are required for normal growth of *Blattella germanica*, but serine is essential only for growth of males (4). In the present work *P. affinis* completed development when the larvae were reared on food lacking any of these four amino acids, although their absence retarded growth. Apparently the larvae needed these amino acids, and they were unable to synthesize them rapidly enough for normal growth.

Growth is not affected by the omission of any of the third group of amino acids, *dl*-aspartic acid, *l*-cysteine, *l*-glutamic acid, *l*-hydroxyproline, and *l*-proline. Generally, these also are dispensable in other insects. Aspartic acid, glutamic acid, hydroxyproline, and proline have not been found necessary for growth in any insect studied (4, 11, 15). In the diet of *B. germanica*, however, proline is necessary for the growth of male nymphs, but not for females (4). A number of insects require cystine (2, 3, 6, 9, 14); in some species, lack of it affects ecdysis or pupation (3, 6, 9, 14). Other species are not greatly affected by the absence of cystine (1, 11, 15). Preliminary assays by the present author have shown that cysteine may be substituted for cystine in the diet of *P. affinis* without effect. The present study shows that cysteine is not necessary for normal growth, ecdysis, or puparial formation.

The present study, therefore, has shown that the dietetic requirements for individual amino acids in the parasite *P. affinis* are similar to those of other insects having different feeding habits. Feeding tests with chemically defined media have established that 10 amino acids are essential for larval growth under aseptic conditions; four others are utilized but are dispensable in the diet, indicating that larvae are able to synthesize them; and five others are not required in the food.

References

1. DAVIS, G. R. F. A nutritional investigation of *Oryzaephilus surinamensis* Linne. Ph.D. Thesis, McGill University, Montreal, Que. 1952.
2. GAY, F. J. A nutritional study of the larvae of *Dermestes vulpinus* F. J. Exptl. Zool. 79 : 93-107. 1938.
3. GOLBERG, L. and DE MEILLON, B. The nutrition of the larvae of *Aedes aegypti* Linnaeus. 4. Protein and amino-acid requirements. Biochem. J. (London), 43 : 379-387. 1948.
4. HILCHEY, J. D. Studies upon the qualitative requirements of *Blattella germanica* (L.) for amino acids under aseptic conditions. Ph.D. Thesis, Cornell University, Ithaca, N.Y. 1951.
5. HINTON, T., NOYES, D. T., and ELLIS, J. Amino acids and growth factors in a chemically defined medium for *Drosophila*. Physiol. Zoöl. 24 : 335-353. 1951.
6. HOUSE, H. L. Nutritional studies with *Blattella germanica* (L.) reared under aseptic conditions. III. Five essential amino acids. Can. Entomologist, 81 : 133-139. 1949.
7. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.) I. A chemically defined medium and aseptic-culture technique. Can. J. Zool. 32 : 331-341. 1954.
8. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). II. Effects of eleven vitamins on growth. Can. J. Zool. 32 : 342-350. 1954.
9. LAFON, M. Recherches sur quelques aspects du besoin qualitatif d'azote. Essai sur le besoin qualitatif d'azote chez un insecte: *Drosophila melanogaster* Meig. Ann. physiol. physicochim. biol. 15 : 215-260. 1939.
10. LECLERCQ, J. Importance de la lysine et du tryptophane dans la nutrition de *Tenebrio molitor* L. Experientia, 4 : 436. 1948.
11. LEMONDE, A. and BERNARD, R. Nutrition des larves de *Tribolium confusum* Duval. II. Importance des acides aminés. Can. J. Zool. 29 : 80-83. 1951.
12. LITCHFIELD, J. T., JR. A method for rapid graphic solution of time-per cent effect curves. J. Pharmacol. Exptl. Therap. 97 : 399-408. 1949.
13. LITCHFIELD, J. T., Jr. and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96: 99-113. 1949.
14. MICHELBACHER, A. E., HOSKINS, W. M., and HERMS, W. B. The nutrition of flesh-fly larvae, *Lucillia sericata* Meig. J. Exptl. Zool. 64 : 109-128. 1932.
15. MOORE, W. Nutrition of *Attagenus* (?) sp. II. (Coleoptera: Dermestidae). Ann Entomol. Soc. Amer. 39 : 513-521. 1946.
16. NOLAND, J. L. and BAUMANN, C. A. Protein requirements of the cockroach, *Blatella germanica* (L.). Ann. Entomol. Soc. Amer. 44 : 184-188. 1951.
17. TRAGER, W. Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. J. Biol. Chem. 176 : 1211-1223. 1948.
18. VAN'T HOOG, E. G. Aseptic culture of insects in vitamin research. Z. Vitaminforsch. 4 : 300-324. 1935.

NUTRITIONAL STUDIES WITH *PSEUDOSARCOPHAGA AFFINIS* (FALL.), A DIPTEROUS PARASITE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.)

IV. EFFECTS OF RIBONUCLEIC ACID, GLUTATHIONE, DEXTROSE, A SALT MIXTURE, CHOLESTEROL, AND FATS[1]

By H. L. House[2]

Abstract

The dipterous parasite *Pseudosarcophaga affinis* (Fall.) reared aseptically on chemically defined media requires ribonucleic acid, dextrose, a salt mixture, cholesterol, and fats for optimum growth; the differences between the results obtained on the experimental media and on the controls were statistically significant. Glutathione is not required for growth, but possibly aids in metamorphosis.

Introduction

Many substances are necessary in the food of insects to provide for their growth. The requirements for these nutrients must be met whenever insects are reared on laboratory diets. This is often difficult, particularly when the diets are chemically defined. Research by many workers has established the identities of a considerable number of these food components and has shown their nutritional value for various insects (Trager (29)).

Nucleic acid, derived from yeast, exerts a beneficial effect on the growth of several species of insects. Ribonucleic acid is important for growth of *Drosophila melanogaster* Mg. reared aseptically on a chemically defined medium (Schultz, St. Lawrence, and Newmeyer (27)). Hinton, Noyes, and Ellis (13) showed that with *D. melanogaster* "higher-than-usual" concentrations of ribonucleic acid partially overcome an inhibition of larval growth caused by high concentrations of certain amino acids.

Subbarow and Trager (28) reported that the polypeptide glutathione improved the growth of at least one species of insect, namely, *Aedes aegypti* (L.), but this was not verified by other work (Golberg, De Meillon, and Lavoipierre (10)). The paucity of information indicates that glutathione has received little attention.

Many insects require a carbohydrate in their food (Fraenkel and Blewett (3); Lafon and Teissier (19)). Usually starch, sucrose, or dextrose is used in the laboratory to fulfill this requirement. But *Phaenicia sericata* (Mg.) grew successfully on an aseptic artificial food containing no carbohydrate (Michelbacher, Hoskins, and Herms (24)). The blowfly *Phormia regina* (Mg.) synthesizes a considerable quantity of carbohydrate from fatty acids

[1] *Manuscript received May 19, 1954.*

Contribution No. 3208, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

during metamorphosis (Haub and Hitchcock (11); Patton, Hitchcock, and Haub (25)). Insects, therefore, differ in their carbohydrate requirements.

Salts can be a limiting factor in the growth of insects. Loeb (23) found that *Drosophila* sp. did not develop in the absence of potassium or phosphorus (as PO_4). A deficiency of calcium retarded the growth of the mosquito *Culiseta incidens* (Thom.) and magnesium sulphate, sodium chloride, and potassium iodide had a similar, though lesser, effect (Frost, Herms, and Hoskins (7)). The growth of *P. sericata* is proportional to the percentage of phosphorus in the diet (Hobson (14)).

It is generally accepted that the lipid requirements of insects can be completely satisfied by several sterols, particularly cholesterol. *P. sericata* grew normally on a medium only when cholesterol was present (Hobson (15)). Similarly, the only fat-soluble material required by *D. melanogaster* is cholesterol, although closely related sterols are effective (Van't Hoog (31)). Cholesterol is an essential growth factor for at least seven species of beetles (Fraenkel and Blewett (4), Gay (8), Leclercq (20)) and three species of moths (Fraenkel and Blewett (4), Fraenkel and Blewett (5), Sarma and Sreenivasaya (26)). Larvae of *A. aegypti* require cholesterol and utilize a number of other sterols (Golberg and De Meillon (9)). Only one insect studied so far requires other lipids: Fraenkel and Blewett (6) found that larvae of *Ephestia kühniella* Zell. require factors contained in wheat-germ oil, one of which is linoleic acid.

House determined the dietetic requirements of the sarcophagid parasite *Pseudosarcophaga affinis* (Fall.) for vitamins (17) and amino acids (18), the larvae being reared aseptically on chemically defined media. In carrying these investigations further, attempts were made to determine the necessity in *P. affinis* for other food components that may be utilized by insects, namely, ribonucleic acid, glutathione, dextrose as a representative carbohydrate, cholesterol as a representative sterol, a salt mixture, and a mixture of fats. The present paper gives the results of these tests.

Materials and Methods

The basic medium, from which the experimental diets are formed by the omission or addition of separate components, has been described by House (16), as have also the techniques for sterilizing the media, obtaining aseptic larvae, and conducting the feeding tests. All tests were replicated three times. The significances of the differences between the results were determined by statistical methods developed by Litchfield and Wilcoxon (22) and Litchfield (21). An index of larval growth (Trager (30)) was also calculated for each medium from the expression n/t, where n is the percentage of larvae reaching the second or third instar within 20 days and t is the mean time in days to reach ecdysis. The greater the value of the expression n/t, the more effective the diet is for promoting growth.

In the present study, in the assays to determine the need for ribonucleic acid, sodium hydroxide was included in the medium as in the other diets to

control its pH. To determine the effects of glutathione this substance was added to the basic medium at 0.001 gm. per 100 ml. of diet. The dietetic effect of cholesterol was determined by withholding cholesterol, but a solution of emulsifying agent, Tween 80 (Atlas Powder Co. Ltd. of Canada, Brantford, Ontario), was included in the medium. The effects of fats were determined by adding 0.75 gm. of domestic shortening or lard to 100 ml. of diet.

Results

The results obtained in the feeding tests are summarized in Table I.

TABLE I

DEVELOPMENT DURING 20 DAYS OF LARVAE OF *P. affinis* WHEN VARIOUS DIETETIC CONSTITUENTS WERE REMOVED OR ADDED TO THE BASIC CHEMICALLY DEFINED MEDIUM; THREE REPLICATES OF APPROXIMATELY 55 EACH

Constituent	Total number of larvae												Pupae from aseptic larvae
	First instar				Second instar				Third instar				
	Max.*	Final†	Dead	Infected	Max.*	Final†	Dead	Infected	Max.*	Final†	Dead	Infected	
Ribonucleic acid	166	8	13	2	143	11	3	7	122	118	2	2	76
Glutathione‡	154	1	7	1	145	8	3	0	134	132	0	2	121
Dextrose	155	1	14	1	139	15	20	3	101	82	17	2	10
Salts	155	14	139	2	0	0	0	0	0	0	0	0	0
Cholesterol	163	2	8	1	152	50	14	5	83	77	4	2	23
Fats‡	153	1	1	0	151	1	1	0	149	147	2	0	131

** Total number of larvae observed, including those that died or became contaminated, in each instar during the assay.*

† Number of living aseptic larvae on the final day of the assay.

‡ Added; all others removed separately.

It is clear that larval growth was affected by some of the components tested. Growth was prevented by the omission of the salt mixture, but it was only retarded by the omission of ribonucleic acid, dextrose, or cholesterol. Apparently glutathione had little effect on growth. The inclusion of a mixture of fats, as contained in domestic shortening or lard, improved the growth of larvae.

Nomographic analysis showed significant differences ($P < 0.05$) (Table II) between the results obtained on the controls and on diets containing fats or those lacking ribonucleic acid, dextrose, the salt mixture, or cholesterol. No difference was found for the food containing glutathione ($P > 0.05$).

The indexes of larval growth for the second and third instars are shown in Table III.

TABLE II

THE PERCENTAGES OF ASEPTIC LARVAE ATTAINING THE THIRD INSTAR AND THE STATISTICAL SIGNIFICANCES OF THEIR RATES OF GROWTH WHEN VARIOUS DIETETIC CONSTITUENTS WERE ADDED OR REMOVED, IN COMPARISON WITH THE CONTROLS

Constituent	Aseptic larvae attaining 3rd instar, %	Slope function* and confidence limits	ET_{50}† and confidence limits, days	Probability P‡	
				Parallelism	Reaction time
Ribonucleic acid	76.9	2.5 (2.2–2.8)	11.1 (10.3–12.0)	> 0.05	<0.05
Control	92.3	2.8 (2.4–3.1)	8.7 (8.2– 9.3)		
Glutathione§	87.4	2.1 (1.9–2.3)	8.8 (8.4– 9.2)	< 0.05	> 0.05
Control	81.2	2.9 (2.5–3.4)	8.8 (8.3– 9.4)		
Dextrose	66.4	2.0 (1.8–2.2)	13.4 (12.5–14.4)	> 0.05	< 0.05
Control	80.6	2.0 (1.8–2.2)	9.5 (9.1–10.0)		
Salts	0	0	0		< 0.05
Control	90.3	1.9 (1.8–2.0)	8.7 (8.4–9.0)		
Cholesterol	52.3	2.5 (2.1–2.9)	14.9 (13.4–16.7)	< 0.05	< 0.05
Control	91.8	1.9 (1.7–2.0)	8.0 (7.7– 8.3)		
Fats§	97.3	2.7 (2.4–3.0)	7.1 (6.9– 7.3)	> 0.05	< 0.05
Control	82.4	2.7 (2.4–3.1)	8.7 (8.2– 9.2)		

* *Equivalent of the standard deviation.*
† *The time required for 50% of the larvae to attain the third instar.*
‡ *Considered significant when less than 0.05.*
§ *Added; all others removed separately.*

TABLE III

INDEXES OF GROWTH OF LARVAE DURING 20 DAYS ON ASEPTIC MEDIA WHEN VARIOUS DIETETIC COMPONENTS WERE ADDED OR REMOVED

Constituent	Second instar			Third instar		
	Larvae attaining instar, % n	Average time, t days	Index n/t	Larvae attaining instar, % n	Average time, t days	Index n/t
Ribonucleic acid	86.4	5.6	15.4	76.9	10.9	7.0
Control	94.8	5.0	19.0	92.3	9.7	9.5
Glutathione*	93.5	4.5	20.8	87.4	9.4	9.3
Control	97.4	4.4	22.1	81.2	9.2	8.8
Dextrose	83.7	4.7	17.8	66.4	12.7	5.2
Control	97.4	4.5	21.6	80.6	9.8	8.2
Salts	0	0	0	0	0	0
Control	96.8	4.6	21.0	90.3	9.7	9.3
Cholesterol	92.9	4.3	21.6	52.3	12.3	4.3
Control	96.8	4.2	23.0	91.8	8.8	10.4
Fats*	98.7	3.9	25.5	97.8	8.5	11.4
Control	97.5	4.2	23.2	82.4	9.1	9.1

* *Added; all others removed separately.*

Discussion

Previous work has determined, with chemically defined media, the necessity for individual vitamins (17) and amino acids (18) for growth of *P. affinis*. The present work shows the effects of other food components. The rate of growth was affected when ribonucleic acid, dextrose, a salt mixture, or cholesterol was omitted from, or when fats were added to, the basic medium. Glutathione added to the food had no effect on the rate of growth.

The omission of ribonucleic acid decreased the rate of growth and the number of larvae reaching the third instar (Table II). The times required for 50% of the larvae to reach the third instar (ET_{50}'s)on the experimental diet and on the control differed by 2.4 days, which is statistically significant ($P < 0.05$). Table I shows that 76 pupae were formed from 118 larvae reared on the diet lacking ribonucleic acid; this is 64.4% as compared with 71.7% from larvae reared on the control. A number of flies were obtained from the pupae. Therefore, ribonucleic acid is required for rapid growth and development, and it affects growth similarly at all stages of development (Table I). Apparently *P. affinis* utilizes ribonucleic acid, or some components of ribonucleic acid and is able to partly satisfy its ribonucleic acid requirement by synthesis. On the other hand, the role of ribonucleic acid in *P. affinis* may be similar to that in *Drosophila melanogaster*, in which this acid overcomes the deleterious effects of high concentrations of certain amino acids on growth (13).

Glutathione had only a slight, if any, effect on growth (Table II). The differences in the reaction times are not significant but the slopes of the growth curves differ significantly. This difference in the slope may be interpreted as evidence that the course of the growth rate was altered (21) by glutathione. The growth of the larvae on the diet containing glutathione was uniform, but slightly longer time was required before any of the larvae reached the third instar; then the population matured more rapidly than the control. Hence, the growth curve for larvae reared on glutathione was steeper and intersected the curve for the controls at the ET_{50} point at 8.8 days. But only a few larvae reared on glutathione failed to mature (Table I) and 121 pupae (91.7%) were formed, more than for the controls. Therefore, if glutathione has an effect on *P. affinis*, it is in its capability to promote more uniform growth and greater survival of larvae, but not necessarily a more rapid rate of growth. The growth indexes of larvae reared on glutathione and on the control (Table III) are high and of the same order. It is concluded that glutathione is not required for the growth of larvae of *P. affinis*, but apparently aids in metamorphosis.

Larvae of *P. affinis* apparently utilize carbohydrate for optimum growth. Both the rate of growth and the number of larvae reaching the third instar were decreased when a representative carbohydrate, dextrose, was omitted from the food (Table II). Mortality of larvae was relatively high during the first and second instars (Table I) and only 10 pupae (12.2%) were obtained from the mature larvae (Table I). It was thought that the high mortality

may have been caused by the lower calorific value of the medium when dextrose was omitted. Feeding tests, however, with a similar medium in which the calorific value of the sugar was supplied by lard did not improve growth. Hence, it appears that *P. affinis* requires a carbohydrate for growth, in spite of the fact that synthesis of this nutrient from fatty acids may take place, as shown in another insect (11, 25).

Omission of the salt mixture from the basal medium prevented growth; none of the larvae developed beyond the first instar (Table I) and the majority died during the assay period. This shows that any mineral impurities contained in the basic diet are inadequate to meet the requirements for growth. Consequently, the medium can be used to determine the salt requirements of *P. affinis*.

Cholesterol is required for the growth of larvae, the effect of its lack becoming most noticeable during the second instar. Table I shows that on a diet lacking cholesterol a large number of larvae developed beyond the first instar, but failed to reach the third. The rate of growth of the larvae was significantly less than for those reared on the control and the number of larvae reaching maturity was only 52.3% (Table III). The significant difference in the slopes of the growth curves of larvae obtained from the experimental and control diets is evidence that omitting cholesterol affected growth. Actually the growth curve of the larvae maturing on the food lacking cholesterol was not as steep as that of the larvae reared on the control. The growth index (Table III) shows that food lacking cholesterol is inefficient in promoting growth. Only 23 (29.9%) of the mature larvae formed pupae (Table I) and only one adult subsequently emerged. None of the pupae survived diapause.

When lard or domestic shortening was added to the basic diet, growth of *P. affinis* improved. The fatty acids contained in lard (Hilditch, Lea, and Pedelty (12)) include those recognized as beneficial dietetic components for certain vertebrates (Burr, Burr, and Miller (2)). Among them is linoleic acid, known to be involved in the development of wing scales and emergence of *Ephestia kühniella* (6). In the present work the addition of lard brought about a greater rate of growth, lower mortality of larvae, and a larger number of mature larvae (Table I). Most of the larvae pupated (89.9%) and many adults were formed. The highest growth index of 11.4 (Table III) indicates that the diet containing fats is the most effective in promoting growth. The results are significantly different from those for the control (Table II).

In previous work (16), matings of flies reared on the basic chemically defined diet did not result in fertilized eggs. Burr and Burr (1) have shown with rats that, in the absence of fats from the diet, sexual development is greatly delayed and reproduction is abnormal or wholly fails. Some of the fatty acids, linolenic and linoleic, are effective in curing this disease (2). It is not yet determined whether these fatty acids may also exert a similar effect on reproduction in *P. affinis*.

These studies clearly indicate the necessity for a number of food components that must be determined more definitely. For example, the necessity for

ribonucleic acid raises the question of its active principles. These may be found among its component nucleotides, purines, and pyrimidines by studies similar to those of Villee and Bissell (32). Similarly, the necessity for individual salts of the salt mixture and for fatty acids contained in lard should be elucidated. The requirements for other carbohydrates and sterols are fields for further investigation in this series of nutritional studies on *P. affinis*.

References

1. BURR, G. O. and BURR, M. M. A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem. 82 : 345-367. 1929.
2. BURR, G. O., BURR, M. M., and MILLER, E. S. On the fatty acids essential in nutrition. III. J. Biol. Chem. 97 : 1-9. 1932.
3. FRAENKEL, G. and BLEWETT, M. The basic food requirements of several insects. J. Exptl. Biol. 20 : 28-34. 1943.
4. FRAENKEL, G. and BLEWETT, M. The sterol requirements of several insects. Biochem. J. (London), 37 : 692-695. 1943.
5. FRAENKEL, G. and BLEWETT, M. The dietetics of the clothes moth, *Tineola bisselliella* Hum. J. Exptl. Biol. 22 : 156-161. 1946.
6. FRAENKEL, G. and BLEWETT, M. Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects, (*Ephestia kühniella, E. elutella, E. cautella* and *Plodia interpunctella* (Lep.)). J. Exptl. Biol. 22 : 172-190. 1946.
7. FROST, F. M., HERMS, W. B., and HOSKINS, W. M. The nutritional requirements of the larva of the mosquito, *Theobaldia incidens* (Thom.). J. Exptl. Zool. 73 : 461-479. 1936.
8. GAY, F. J. A nutritional study of the larva of *Dermestes vulpinus* L. J. Exptl. Zool. 79 : 93-107. 1938.
9. GOLBERG, L. and DE MEILLON, B. The nutrition of larva of *Aedes aegypti* Linnaeus. 3. Lipid requirements. Biochem. J. (London), 43 : 372-379. 1948.
10. GOLBERG, L., DE MEILLON, B., and LAVOIPIERRE, M. The nutrition of the larva of *Aedes aegypti*. II. Essential water-soluble factors from yeast. J. Exptl. Biol. 21 : 84-89. 1945.
11. HAUB, J. G. and HITCHCOCK, F. A. The interconversion of foodstuffs in the blowfly (*Phormia regina*) during metamorphosis. Ann. Entomol. Soc. Amer. 34 :32-37. 1941.
12. HILDITCH, T. P., LEA, C. H., and PEDELTY, W. H. The influence of low and high planes of nutrition on the composition and synthesis of fat in the pig. Biochem. J. (London), 33 : 493-504. 1939.
13. HINTON, T., NOYES, D. T., and ELLIS, J. Amino acids and growth factors in a chemically defined medium for *Drosophila*. Physiol. Zoöl. 24 : 335-353. 1951.
14. HOBSON, R. P. Growth of blow-fly larvae on blood and serum. II. Growth in association with bacteria. Biochem. J. (London), 29 : 1286-1291. 1935.
15. HOBSON, R. P. On a fat-soluble growth factor required by blow-fly larvae. II. Identity of the growth factor with cholesterol. Biochem. J. (London), 29 : 2023-2026. 1935.
16. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). I. A chemically defined medium and aseptic-culture technique. Can. J. Zool. 32 : 331-341. 1954.
17. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). II. Effects of eleven vitamins on growth. Can. J. Zool. 32 : 342-350. 1954.
18. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). III. Effects of nineteen amino acids on growth. Can. J. Zool. 32 : 351-357. 1954.
19. LAFON, M. and TEISSIER, G. Les besoins nutritifs de la larve de *Tenebrio molitor*. Compt. rend. soc. biol. 131 : 75-77. 1939.
20. LECLERCQ, J. Sur les besoins en stérols de larves de *Tenebrio molitor* L. Biochim. et Biophys. Acta, 2 : 614-617. 1948.
21. LITCHFIELD, J. T., JR. A method for rapid graphic solution of time-per cent effect curves. J. Pharmacol. Exptl. Therap. 97 : 399-408. 1949.

22. LITCHFIELD, J. T., JR. and WILCOXON, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96 : 99-113. 1949.
23. LOEB, J. The salts required for the development of insects. J. Biol. Chem. 23 : 431-434. 1915.
24. MICHELBACHER, A. E., HOSKINS, W. M., and HERMS, W. B. The nutrition of flesh-fly larvae, *Lucilia sericata* Meig. J. Exptl. Zool. 64 : 109-128. 1932.
25. PATTON, M. B., HITCHCOCK, F. A., and HAUB, J. G. The interconversion of foodstuffs in the blowfly (*Phormia regina*) during metamorphosis. Ann. Entomol. Soc. Amer. 34 : 26-31. 1941.
26. SARMA, P. S. and SREENIVASAYA, M. Studies in insect nutrition: The nature of the fat-soluble factor. Current Sci. (India), 10 : 525-526. 1941.
27. SCHULTZ, J., ST. LAWRENCE, P., and NEWMEYER, D. A chemically defined medium for the growth of *Drosophila melanogaster*. Anat. Record, 96 : 540. 1946.
28. SUBBAROW, Y. and TRAGER, W. The chemical nature of growth factors required by mosquito larvae. II. Pantothenic acid and vitamin B_6. J. Gen. Physiol. 23 : 561-568. 1940.
29. TRAGER, W. Insect nutrition. Biol. Revs. Cambridge Phil. Soc. 22 : 148-177. 1947.
30. TRAGER, W. Biotin and fat-soluble materials with biotin activity in the nutrition of mosquito larvae. J. Biol. Chem. 176 : 1211-1223. 1948.
31. VAN'T HOOG, E. G. Aseptic culture of insects in vitamin research. Z. Vitaminforsch. 5 : 118-126. 1936.
32. VILLEE, C. A. and BISSELL, H. B. Nucleic acids as growth factors in *Drosophila*. J. Biol. Chem. 172 : 59-66. 1948.

HELMINTH PARASITES OF REPTILES, BIRDS, AND MAMMALS IN EGYPT

I. *STREPTOPHARAGUS KUNTZI* SP. NOV., FROM RODENTS, WITH A REVIEW OF THE GENUS[1]

By Betty June Myers[2]

Abstract

A new species of nematode, *Streptopharagus kuntzi*, is described from rodents in Egypt and the genus *Streptopharagus* is reviewed.

During a routine examination of helminths from North African hosts collected under the direction of Dr. Robert Kuntz (United States Naval Medical Research Unit No. 3, Cairo, Egypt), a number of specimens belonging to the genus *Streptopharagus* were found from murine rodents. A study of these helminths has shown them to constitute a species new to science. The author wishes to thank Dr. Kuntz for making these specimens available for study.

Streptopharagus kuntzi sp. nov.

Specific Diagnosis

The males measure 9.6 to 25.6 mm. in length and 0.19 to 0.48 mm. in maximum thickness; the females measure 19.2 to 44.2 mm. in length and 0.28 to 0.65 mm. in maximum thickness.

The cuticle is transversely striated. Cervical alae or cervical expansions are lacking. The lips are trilobed and reduced. The mouth bears six teeth, and a dorsal and ventral tooth are present at the anterior end of the pharynx.

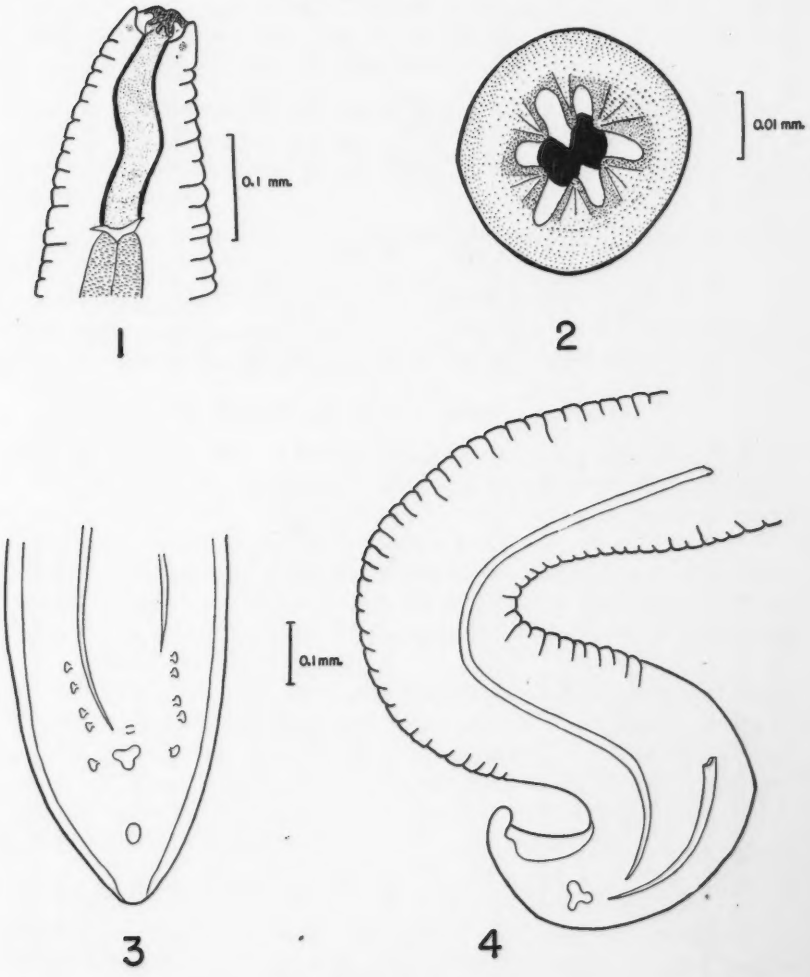
The pharynx is 0.13 to 0.22 mm. in length in the male and 0.13 to 0.24 mm. in the female, and forms the characteristic half-spiral turn about the middle of its length. The walls of the pharynx show transverse ridges; dorsal and ventral pharyngeal teeth are present. The pharynx is followed by a long esophagus measuring 2.0 to 3.64 mm. in the male and 2.40 to 5.0 mm. in the female. The nerve ring is located 0.21 to 0.48 mm. from the anterior end in the male and 0.24 to 0.59 mm. in the female. The excretory pore is at the level of the nerve ring.

The caudal alae of the male are transversely straited. Four pairs of preanal and one pair of postanal pedunculate papillae are present, and a single sessile papilla is present near the tip of the tail.

[1] *Manuscript received May 31, 1954.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada, with financial help from the National Research Council of Canada. This survey has been made possible by the co-operation of Dr. R. E. Kuntz, Mr. Harry Hoogstraal, and the technical assistance of Mr. G. M. Malakatis, all of the United States Naval Medical Research Unit No. 3, Cairo, Egypt.

[2] *Junior Research Assistant.*



FIGS. 1-4. *Streptopharagus kuntzi.*

FIG. 1. Anterior extremity, ventral view.
FIG. 2. Head end, *en face* view, showing stomal and pharyngeal teeth.
FIG. 3. Posterior extremity of male, ventral view, showing papillae pattern.
FIG. 4. Posterior extremity of male, lateral view, showing spicules and gubernaculum.

The spicules are unequal and dissimilar, the left one measuring 0.96 to 1.80 mm. in length and the right one, 0.26 to 0.33 mm. in length. The gubernaculum is asymmetrical, measuring 0.026 to 0.033 mm. The tail of the male measures 0.19 to 0.37 mm. in length.

The vulva of the female is small and non-salient, situated 7.2 to 16.9 mm. from the anterior end. The vagina is muscular, beginning as an ovejector

TABLE I

MEASUREMENTS OF *Streptopharagus kuntzi* ACCORDING TO HOST

		Hosts			
		Meriones 2 male 3 female	*Acomys** 2 female	*Rattus* 5 male 6 female	*Gerbillus* 5 male 4 female
Length	Male	9.6 to 11.2	—	11.2 to 27	18.5 to 25.7
	Female	19.2 to 24	19.2 to 20.8	20.8 to 29.8	40.4 to 44.2
Width	Male	0.19 to 0.24	—	0.25 to 0.48	0.36 to 0.39
	Female	0.30 to 0.32	0.18 to 0.2	0.28 to 0.48	0.52 to 0.65
Distance from anterior to:					
end of pharynx	Male	0.19 to 0.21	—	0.13 to 0.22	—
	Female	0.19	—	0.19 to 0.21	—
excretory pore	Male	Level of nerve ring	Level of nerve ring	Level of nerve ring	Level of nerve ring
nerve ring	Male	0.21	—	0.25 to 0.32	0.32 to 0.48
	Female	0.24	0.21	0.27 to 0.36	0.45 to 0.59
end of esophagus	Male	2.08 to 2.4	—	2.08 to 3.2	2.16 to 3.64
	Female	2.72 to 3.3	2.72	2.4 to 3.4	4.27 to 5
vulva		7.2	7	7.8 to 10	16.31 to 16.9
Spicule length	Left	1	—	0.96 to 1.10	1.56 to 1.83
	Right	0.33	—	0.27 to 0.33	0.26 to 0.33
Gubernaculum		0.030	—	0.030 to 0.036	0.026 to 0.031
Tail length	Male	0.19 to 0.21	—	0.18 to 0.19	0.18 to 0.37
	Female	0.19	—	0.19 to 0.21	0.16 to 0.38
Ova		30-36μ by 15-17μ	30-33μ by 15-18μ	33-36μ by 15-18μ	28-43μ by 18-22μ

* *These specimens were badly contracted and only a few measurements could be made.*

Note: *Unless otherwise noted all measurements are in millimeters.*

which runs posteriorly and divides into two uterine branches. The tail of the female measures 0.16 to 0.38 mm. in length. The eggs measure 28 to 43μ by 15 to 22μ, and are embryonated when laid.

Host: *Rattus* sp., *Gerbillus* sp., *Acomys cahirinus*, *Meriones libycus* (type host).
Location: Stomach and large intestine.
Locality: Egypt.

Type specimens will be deposited in the Helminthological Collection of the United States National Museum.

The species is named in honor of Dr. Robert Kuntz.

Table I gives a tabulation of the measurements of *S. kuntzi* according to host. Unless otherwise indicated all measurements are in millimeters.

A Review of the Genus *Streptopharagus*

The last review of the genus *Streptopharagus* was that of Ortlepp in 1925 (9); in view of the time that has elapsed and the present confusion regarding the species of this genus, it seems desirable to resurvey the genus at this time and to attempt to clarify the position of its species.

In 1912 Blanc (4) established the genus *Streptopharagus*, with *S. armatus* as the genotype, for nematodes collected from *Macacus cynomologus*. Blanc did not, either then or later, present a separate diagnosis of morphological characteristics for the genus.

Baylis (1), in 1923, and York and Maplestone (16), in 1926, patterned their generic diagnoses on Blanc's description. In 1925, Ortlepp (9) reviewed the genus but did not include a generic diagnosis. In 1926, Baylis and Daubney (3) and, in 1939, Baylis (2) formulated brief generic diagnoses. To establish a generic diagnosis for the genus, the following description, which is based on but expanded from that of Baylis (2), is suggested.

Genus Streptopharagus Blanc, 1912

Lips trilobed, greatly reduced. An inflation of the cervical cuticle may be observed. Mouth hexagonal with six simple or complex teeth. Buccal capsule continuous posteriorly with a thick-walled tubular pharynx. Pharynx forms a half spiral in its course. Dorsal and ventral teeth present at the anterior end of the pharynx. Esophagus consisting of a short muscular anterior portion and a partly glandular posterior portion. Caudal end of the male spirally coiled with broad alae. Four pairs of preanal and one pair of postanal pedunculate papillae present. Spicules unequal and dissimilar. A small accessory piece present. Vulva in front of the middle of the body. Adult worms in the stomach or intestine of mammals.

Genotype: *Streptopharagus pigmentatus* (von Linstow, 1897), Railliet and Henry, 1918.

Characteristics such as the inflation of the cervical cuticula and presence of claw-like cloacal processes on the tail of the male are regarded by most authors as variable characteristics depending upon fixation.

Six species (including *S. kuntzi*) of the genus *Streptopharagus* have been described, two from primates, and four from rodents and carnivores.

Rodent Species

Seurat (13) erected *S. numidicus* to contain specimens which he described from the fennec fox (*Vulpes cerdo*) from North Africa. As his specimens contained only immature forms some authors consider the erection of a new species unjustified. Ortlepp (9) referred two specimens from *Gerbillus pygarus*, Egypt, to this species, on the basis of spicule size and total length; again, Ortlepp's specimens were immature forms and in a poor state of preservation. It is possible that the fox is only an accidental host and that *Gerbillus*, or a related rodent, is the normal host. The fox could obtain an accidental infection by preying on an infected rodent. In view of these facts the author is of the opinion that until more information is available, both Seurat's and Ortlepp's specimens should be considered as *Streptopharagus* sp. inq.

Baylis (1) described *S. sudanensis* from *Gerbillus gerbillus*. This species appears to be closely related to *S. numidicus* and differs from it only in total length, spicule and gubernaculum size, and the more anterior position of the vulva. Until the true host for *S. numidicus* is established or additional material examined and described, however, it is impossible to regard these species as synonymous.

Le Roux (6) described *S. geosciuri* from *Geosciurus capensis* from Africa. Le Roux's measurements of the spicules in this species are comparable with those of *S. numidicus* described by Ortlepp (9) from *Gerbillus pygarus*, and those of the same species described by Seurat (13) from *Vulpes cerdo*. Le Roux described *S. geosciuri* in detail and related the position of the internal organs to the cervical papillae; the latter structures have not been mentioned by previous authors as being present in rodent material. Cervical papillae were not observed by the author in her examination of specimens of *S. kuntzi*.

Except for measurements, most descriptions of species of *Streptopharagus* have been very brief with few specific characteristics mentioned. Accordingly, until more material of the species previously described has been examined the present situation must remain, even although most workers are not convinced that measurements alone are sufficient to validate species differentiation.

Table II gives the measurements for the four species of *Streptopharagus* described from rodents. *S. numidicus* Seurat, 1917, from the fennec fox (*Vulpes cerdo*) is included in order that the measurements for this species may be compared with those of the two specimens obtained from *Gerbillus pugarus* referred to this species by Ortlepp (9) in 1925.

TABLE II

MEASUREMENTS FOR THE FOUR SPECIES OF *Streptopharagus* DESCRIBED FROM RODENTS FROM NORTH AFRICA

		Species and hosts			
		S. numidicus	*S. sudanensis*	*S. geosciuri*	*S. kuntzi*
		Vulpes cerdo	*Gerbillus gerbillus*	*Geosciurus capensis*	*Rattus* sp. *Meriones libycus* *Acomys cahirinus* *Gerbillus* sp.
Length	Male	13.9 (13)*	14 to 20	12 to 16	9.6 to 25.6
	Female	16.8 (14)*	Up to 41	24 to 33	19.2 to 44.2
Width	Male	0.385	0.2 to 0.4	0.215 to 0.250	0.12 to 0.48
	Female	0.600	1.0	0.246 to 0.415	0.28 to 0.65
Distance from anterior to:					
end of pharynx	Male	0.16	0.12 to 0.2	—	0.13 to 0.22
	Female	0.18	0.24	—	0.11 to 0.16
nerve ring	Male	—	0.28 to 0.44	In front of left cervical papillae	0.21 to 0.48
	Female	—	—		0.24 to 0.50
excretory pore		—	Behind nerve ring	Level of right cervical papillae	Approximate level of nerve ring
end of esophagus	Male	2.5	2.08 to 2.90	1.72 to 2.1	1.3 to 3.6
	Female	2.8	3.2	2.1 to 2.6	1.3 to 5.0
vulva		7.7	8 to 10	7.5 to 10	7.2 to 16.9
Spicule length	Left	1.9 (2.2)*	2.3	2.23 to 2.62	0.96 to 1.8
	Right	0.42 (0.48)*	0.59	0.416 to 0.448	0.27 to 0.33
Gubernaculum		0.07	0.44	0.054 to 0.064	0.026 to 0.033
Tail length	Male	—	0.2 to 0.32	—	0.018 to 0.033
	Female	0.38	0.3	—	0.16 to 0.38
Ova		—	0.05 × 0.035	35.2 × 17.6μ	30-40μ by 15.22μ

* *Ortlepp (9) refers two specimens from Gerbillus pygarus to this species on the basis of these measurements.*

Note: ***Unless otherwise noted all measurements are in millimeters.***

Primate Species

In 1939 Baylis (2) made *S. pigmentatus* (von Linstow, 1897) Railliet and Henry, 1918, genotype, with *Spiroptera pigmentatus* von Linstow, 1897, *Streptopharagus armatus* Blanc, 1912, *S. intermedius* Ortlepp, 1925, and *S. magnus* Maplestone, 1929, as synonyms. Baylis did not mention *S. baylisi* Ortlepp, 1925, which was established for *S. armatus* Blanc of Baylis, 1923. After studying Ortlepp's work the author considers it desirable to retain *S. baylisi* as a valid species on the basis of spicule size, the hooked condition of the tip of the right spicule, and a series of prominent claw-like cloacal structures with hooked tips.

The presence or absence of the claw-like cuticular structure of the male cloacal region has been a subject of much discussion by many authors. Ortlepp (9) failed to find these structures in *S. armatus*, *S. pigmentatus*, and *S. intermedius*, but records them as being present in *S. baylisi*. Sandground (11) considered the separation of *S. baylisi* on the presence of these claw-like structures to be well founded. Le Roux (6) showed that the presence of these structures was a result of the folding of the ventral cuticula while the caudal alae were being oriented under a cover slip. He was of the opinion

TABLE III

MEASUREMENTS FOR THE TWO SPECIES OF *Streptopharagus* DESCRIBED FROM PRIMATES

		S. baylisi	*S. pigmentatus**
Length	Male	35 to 39	22 to 55
	Female	39 to 46	32 to 70
Width	Male	0.7	0.6 to 1.0
	Female	0.87	0.8 to 1.5
Distance from anterior to:			
end of pharynx		0.25 to 0.27	3 to 6
nerve ring	Male	—	0.55 to 0.68
	Female	0.6	0.60 to 0.90
excretory pore		0.7 to 0.75	0.44 to 0.7
end of esophagus	Male	6.6 to 7.1	6.6 to 7.1
	Female	9.0	6.8 to 12
vulva		11.76 to 19.0	9.5 to 20
Spicule length	Left	10 to 12	4 to 6.7
	Right	0.55	0.5 to 0.75
Gubernaculum		0.07	0.033 to 0.078
Tail length	Male	0.07	0.4 to 0.5
	Female	0.35	0.4 to 0.9
Ova		0.035 × 0.0125	0.03 to 0.042 × 0.0173 to 0.28

* *Based on descriptions by Baylis (1, 2), Blanc (4), Mönnig (8), Ortlepp (9), Sandground (11), and Vevers (15).*

Note: *Unless otherwise noted all measurements are in millimeters.*

that their presence in preserved specimens could be due to contraction of certain muscles or to pressure applied when the specimens were being flattened. Baylis (2) reported the presence of these cuticular structures and considered them to represent a special local development of the tessellated pattern of the general surface of this region. Baylis was critical of Sandground's (11) description of the appearance of "claw-like structures". After surveying the literature the writer concludes that it is possible that these structures are highly variable in development and that they are found only in primate material. If this is true, they could be used to separate primate species from those occurring in rodents.

Table III shows the measurements of the species of *Streptopharagus* reported from primates. All measurements are in millimeters.

Table IV indicates the host and geographic distribution of *Streptopharagus* occurring in primates.

TABLE IV

HOST AND GEOGRAPHIC DISTRIBUTION OF *Streptopharagus* OCCURRING IN PRIMATES

Species	Host	Locality
S. pigmentatus	*Cercopithecus albigularis*	Africa (7)
	C. diana	Dan River, Liberia (12)
	C. patas	? (9)
	C. pygerythrus	Transvaal (8)
	Hylobata hooklock	India*
	H. leucogenys	Indo-China (12)
	Macaca sp.	Belgian Congo (10)
	Macaca sp.	Indo-China (12)
	M. cynomologus	India (?) (4)
	M. nemestrinus	India (15)
	M. sinica auriformis	Ceylon (5)
	M. rhesus	Hagensbeck Tierpark (14)
	Papio hamadryas†	Hagensbeck Tierpark (14)
	P. hamadryas	Abyssinia (9)
	P. porcaries	Transvaal (8)
S. baylisi	*P. hamadryas*	Tanganyika (9)
	P. langheldi	Abyssinia (9)

* *Exact locality of the host was not given. Reported by York and Maplestone, 1926 (not available). Reference to this was in Baylis (2).*

† *Travassos and Vogelsang (14) list this as H. hamadryas.*

Key to the Species of *Streptopharagus*

1. Parasites of primates..... 2
   Parasites of rodents or carnivores..... 3

2. Left spicule long, over 11.0 mm., tip of the right spicule hooked..... *S. baylisi*
   Left spicule less than 8 mm., tip of the right spicule straight..... *S. pigmentatus*

3. A single sessile median papillae at the anterior border of the cloacal aperture and a pair of sessile papillae behind the anus..... *S. geosciuri*
   A single sessile papillae near the tip of the tail..... *S. kuntzi*
   A group of five pairs of sessile subventral papillae near the tip of the tail.... *S. sudanesis*

References

1. BAYLIS, H. A. On the nematode genus *Streptopharagus* with some remarks on the genus *Spirocerca*. Trans. Roy. Soc. Trop. Med. Hyg. 16: 486-497. 1923.
2. BAYLIS, H. A. Fauna of British India (including Ceylon and Burma). Vol. 2. Nematodes. Secretary of State for India, London. 1939.
3. BAYLIS, H. A. and DAUBNEY, R. A synopsis of the families and the genera of nematodes. British Museum, London. 1926.
4. BLANC, G. R. Un nématode nouveau *Streptopharagus armatus* (n. gen., n. sp.), parasite du macaque (*Macacus cynomolgus*). (Note préliminaire.) Compt. rend. soc. biol. 72: 456-457. 1912.
5. CRUZ, H. Observations on some spirurid nematodes from Ceylon vertebrates. Ceylon J. Sci. B, 24: 131-134. 1950.
6. LE ROUX, P. L. A spirurid (*Streptopharagus geosciuri* sp. nov.) from the stomach of the cape ground squirrel (*Geosciurus capensis*). Rept. Director Vet. Services Animal Ind. Onderstepoort, 16: 201-204. 1930.
7. LINSTOW, O. VON. Zur Systematik der Nematoden nebst beschreibung neuer Arten. Arch. mikroskop. Anat. Entwicklungsmech. 49: 608-622. 1897.
8. MÖNNIG, H. O. South African parasitic nematodes. 9th-10th Rept. Director Vet. Education & Research, Onderstepoort, 446-448. 1924.
9. ORTLEPP, R. J. A review of the members of the genus *Streptopharagus* Blanc, 1912. J. Helminthol. 3: 209-216. 1925.
10. RAILLIET, A. and HENRY, A. Nématodes parasites du Congo Belge. Bull. soc. pathol. exotique, 11: 82-86. 1918.
11. SANDGROUND, J. H. Notes and descriptions of some parasitic helminths collected by the expedition. Rept. Harvard-African Expedition to the African Republic of Liberia and the Belgian Congo. Contribs. Dept. Trop. Med. and Inst. Trop. Biol. and Med. (Harvard University), 5: 462-486. 1930.
12. SANDGROUND, J. H. Report on the nematode parasites collected by the Kelly-Roosevelt Expedition to Indo-China, with descriptions of several new species. Z. Parasitenk. 5: 542-583. 1933.
13. SEURAT, L. G. Sur les Spiroptères des carnivores du Nord Africain. Bull. soc. hist. nat. Afrique Nord, 8: 21-24. 1917.
14. TRAVASSOS, L. P. and VOGELSANG, E. G. Mitteilung über das Genus *Streptopharagus* Blanc, 1912. Zool. Anz. 85: 110-111. 1929.
15. VEVERS, G. M. On the parasitic nematoda collected from mammalian hosts which died in the Gardens of the Zoological Society of London during the years 1919-1921, with a description of three new genera and three new species. Proc. Zool. Soc., London (1922), 61: 901-919. 1923.
16. YORK, W. and MAPLESTONE, P. A. The nematode parasites of vertebrates. J. & A. Churchill, Ltd., London. 1926.

Notes to Contributors

Manuscripts

(i) *General*

Manuscripts should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables (each typed on a separate sheet) and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts should be carefully checked before they are submitted; authors will be charged for changes made in the proof that are considered excessive.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Numerous small tables should be avoided.

Illustrations

(i) *General*

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard.

As many photographs as possible should be mounted together (with a **very** small space between each photo) to reduce the number of cuts required. Full use of the space available should be made and the ratio of height to width should correspond to that of a journal page; however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. × 11 in.) and making allowance for illustrations (not inserts). The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents